Genomics and precision medicine

Outline

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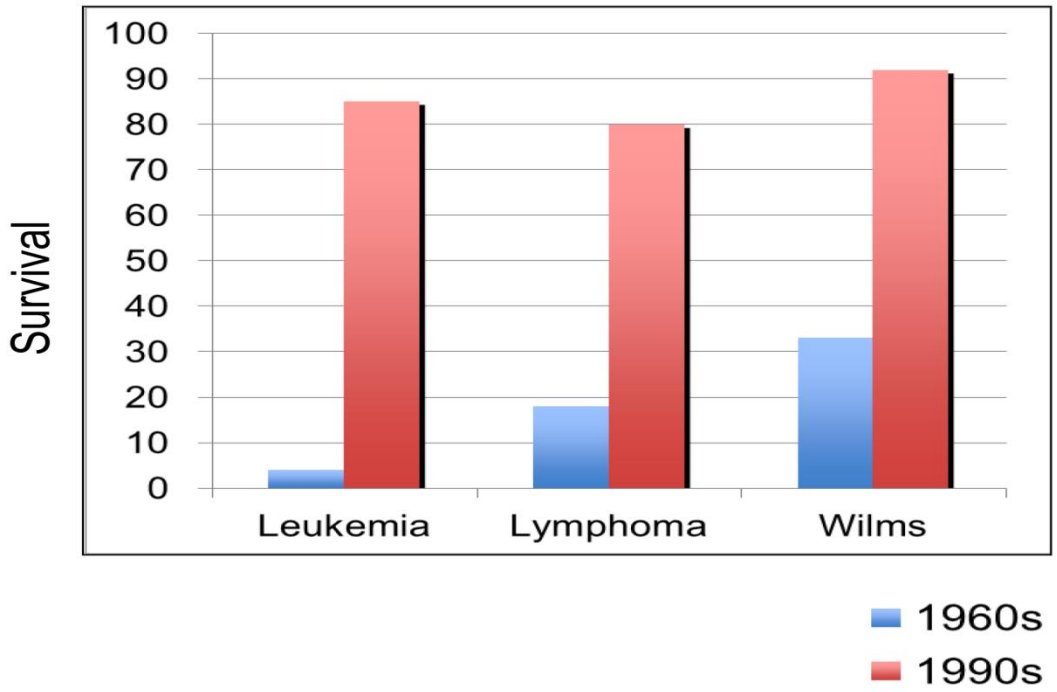
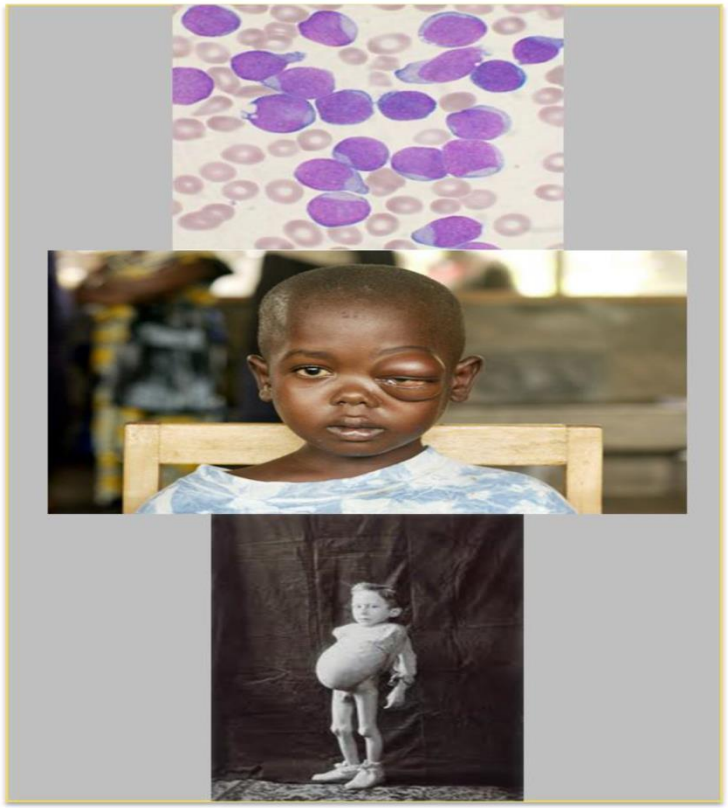
- Success and Challenges of Treating Pediatric Cancers
- Genomics
- Next-generation Sequencing
- Application of next-generation sequencing:
  - Diagnosis
  - Identification of molecular target
- Precision Therapy

Childhood cancer

National Cancer Institute

Childhood cancer: The beginning of a modern medical success story



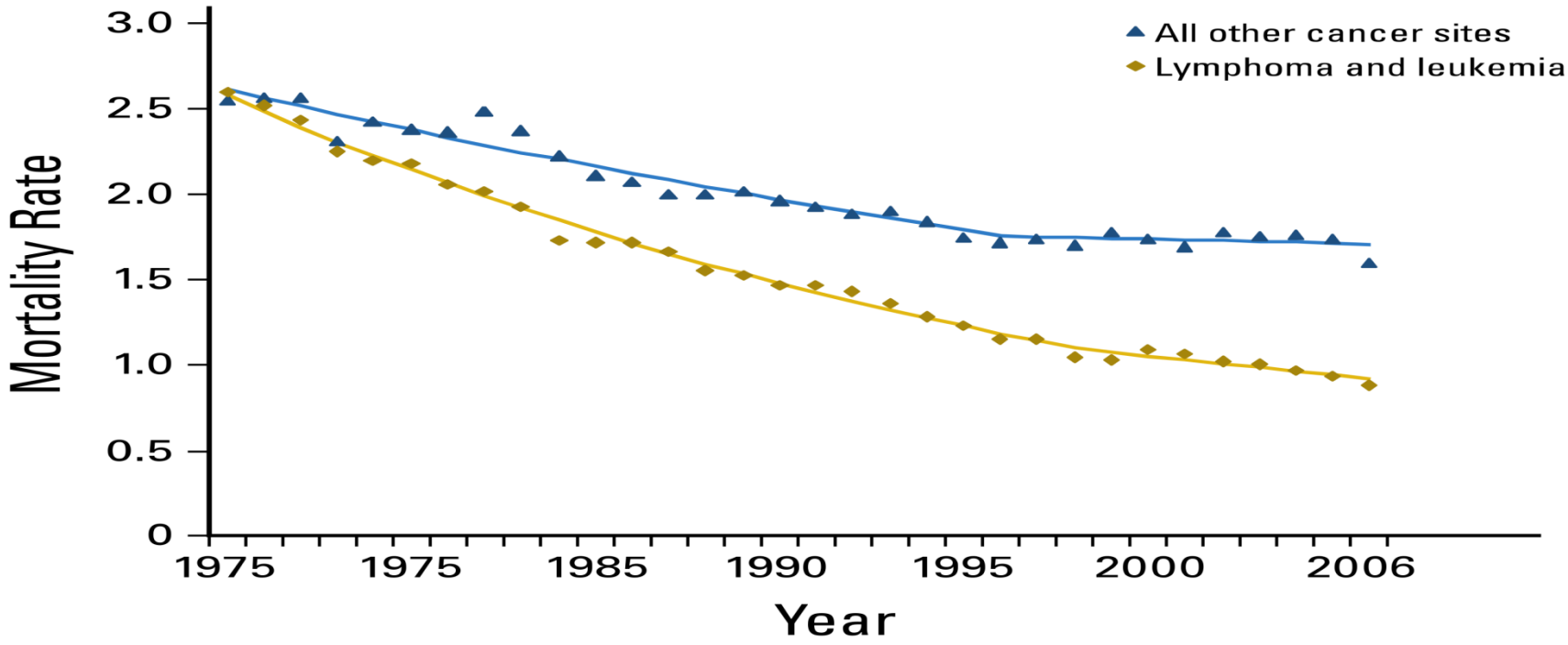


Courtesy: John Maris

Mortality rates

National Cancer Institute

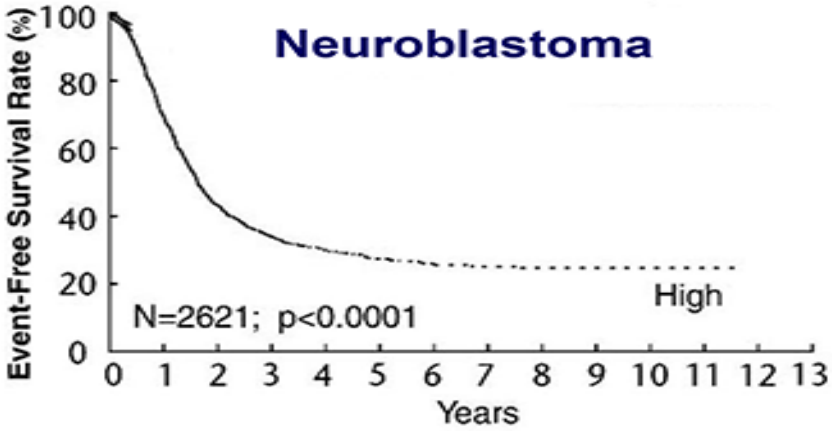
However in the past 16 years no improvement in mortality rates despite increased intensity of treatment

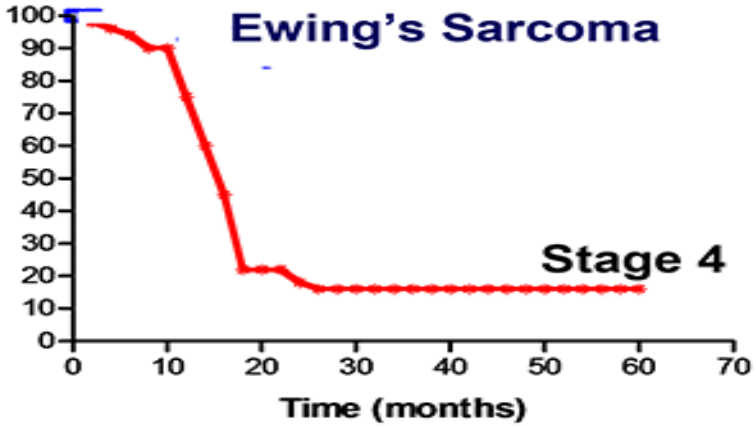


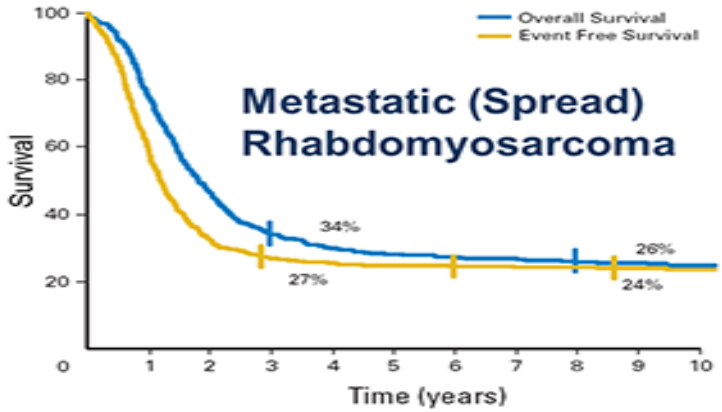
Courtesy: Malcolm Smith

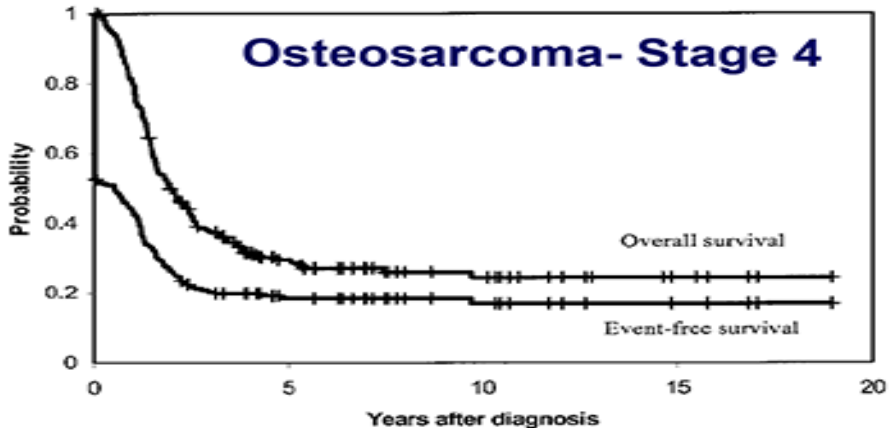
Pediatric cancers

Metastatic, Recurrent, & Refractory Disease Remains Incurable









Gene expression

The dramatic consequences of gene expression in biology

Anise swallowtail, *Papilio zelicaon*

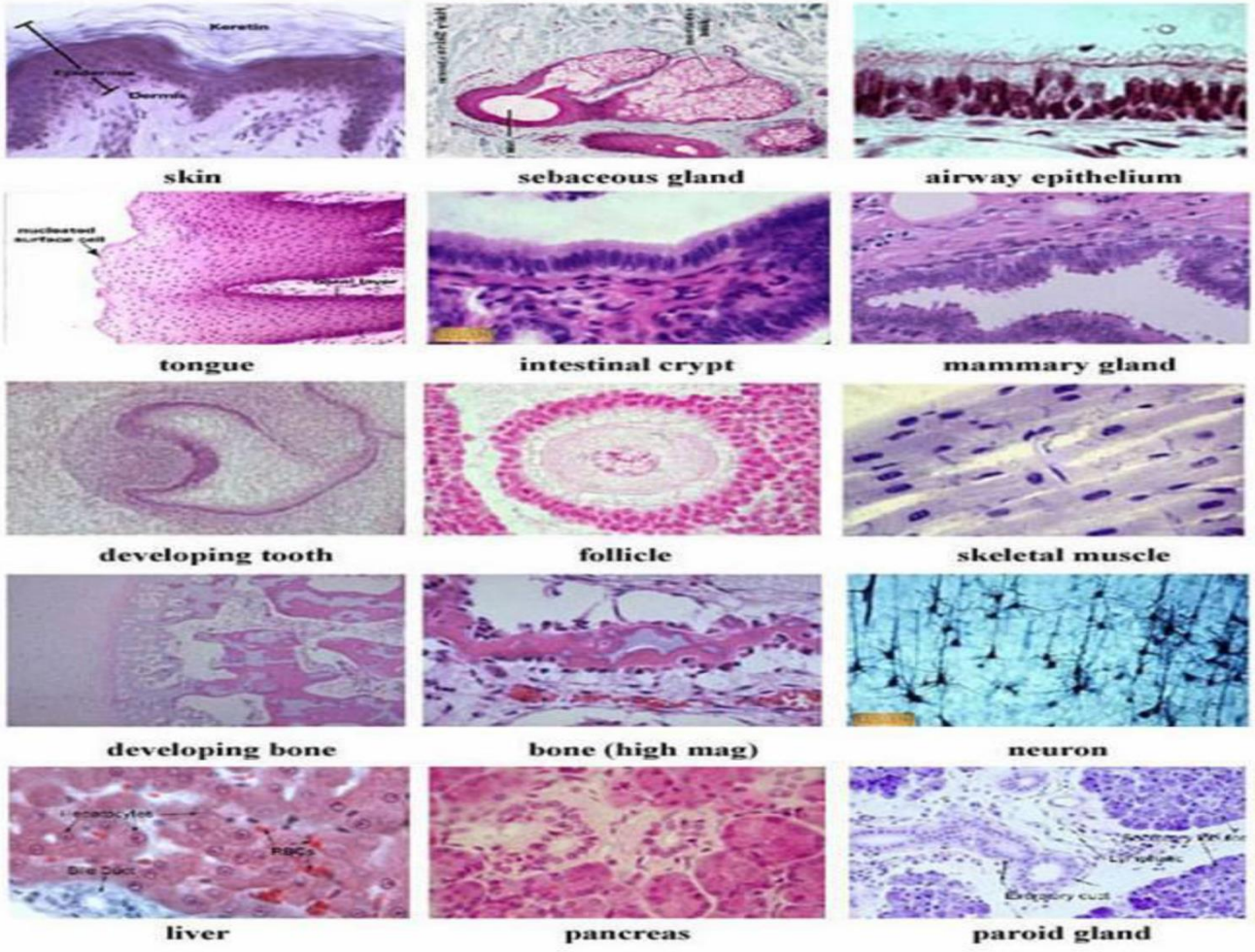
Same genome →
Different expression pattern
Different proteome
Different tissues
Different physiology

Gene expression

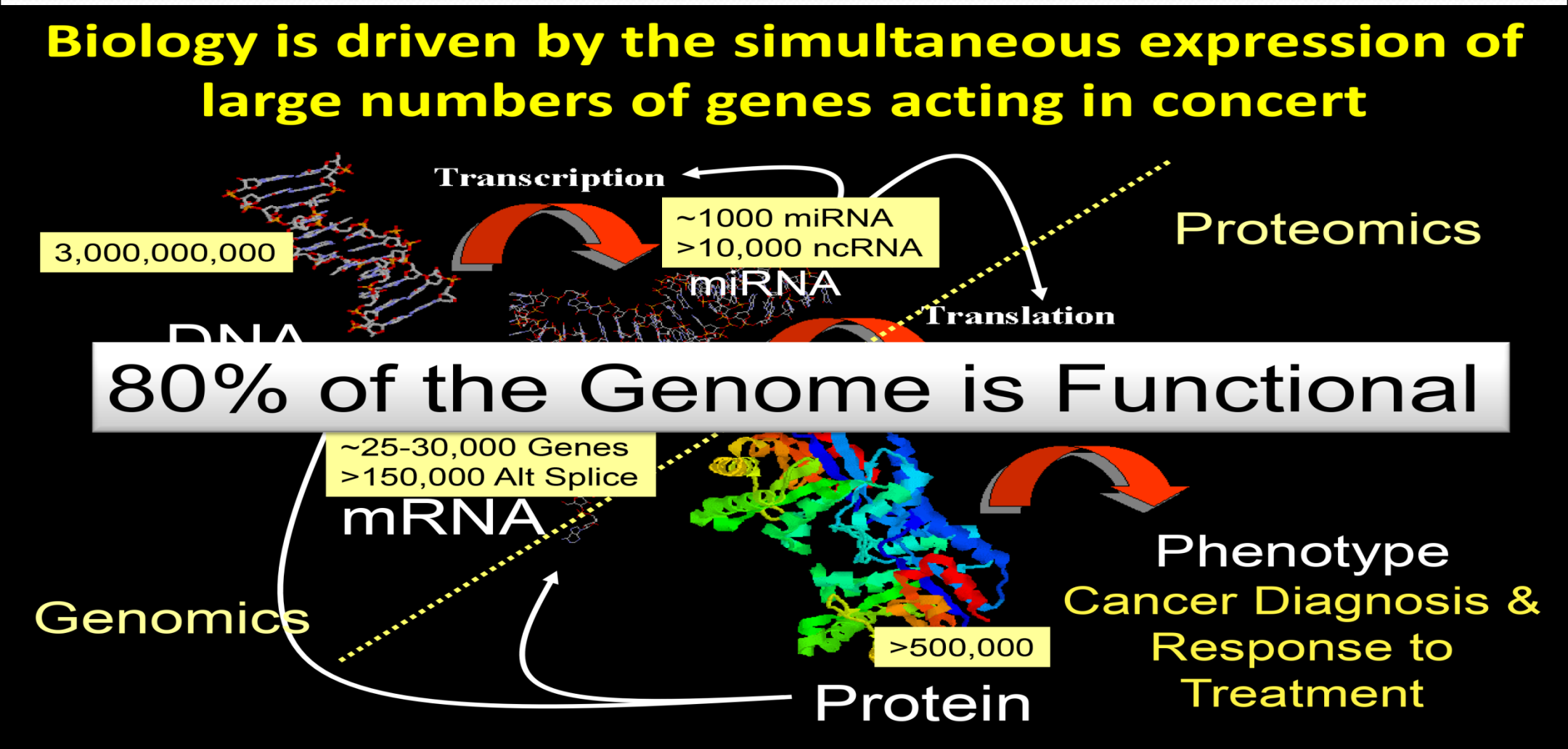
...but the complexity and diversi

Same genome or DNA →

- Different expression pattern
- Different proteome
- Different tissues
- Different physiology



Gene expression

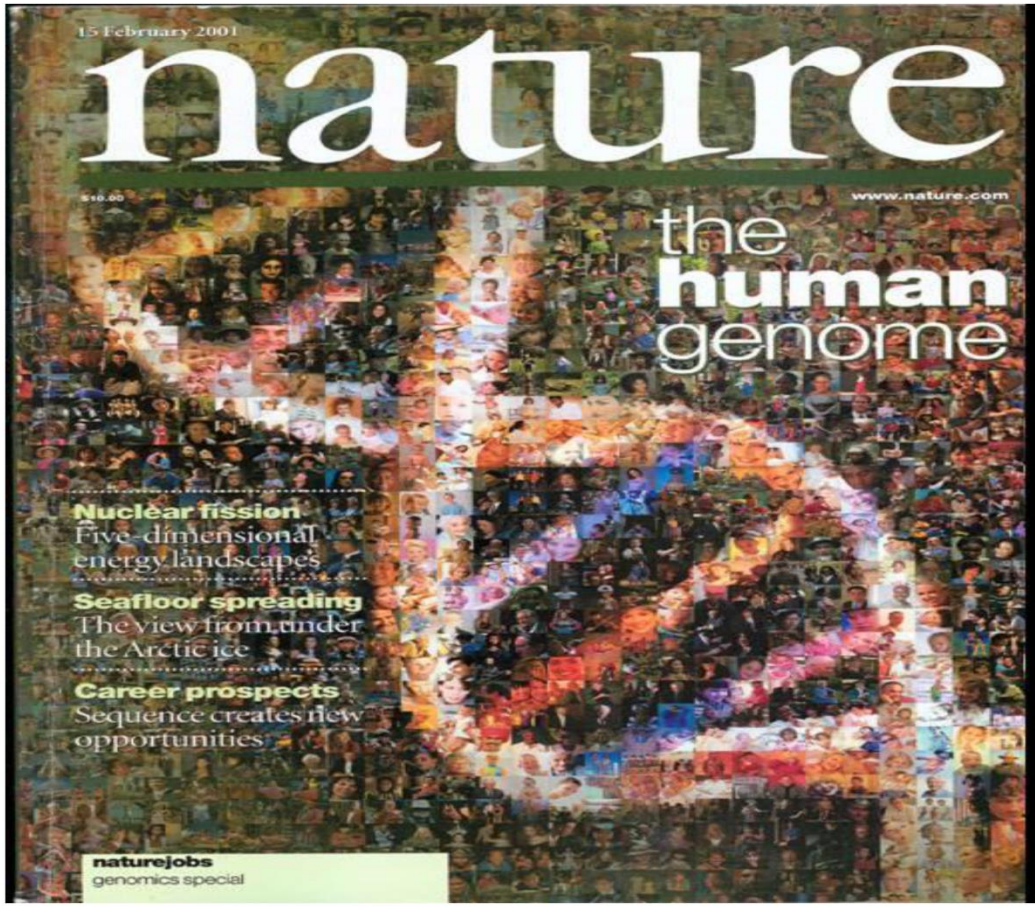


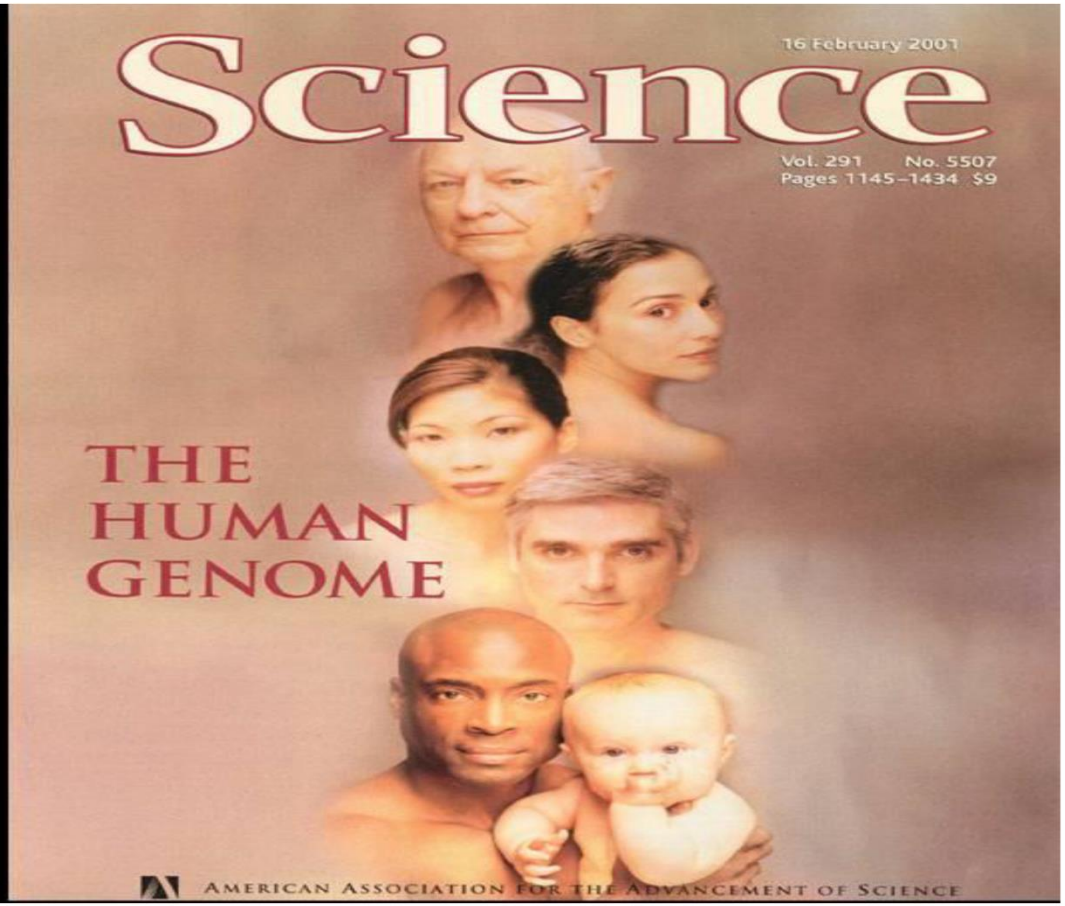
Gene measurement

Challenge: how to measure/detect genes and their products in a massively parallel way?

- **High-throughput technologies**
- **Computational power**

Human genome





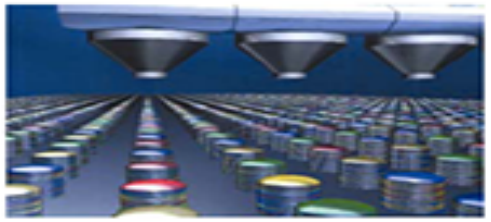
Microarrays

1st generation genomic tool: microarrays

Printing microarrays

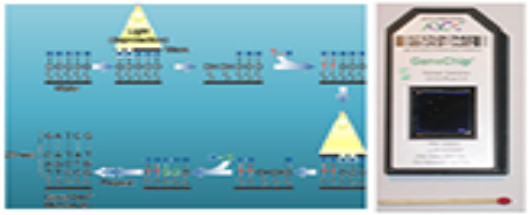
Mechanical

Electronic Piezo

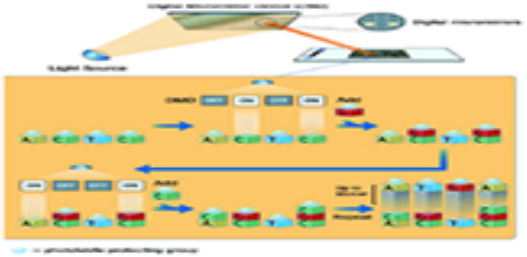


In-situ synthesis microarrays

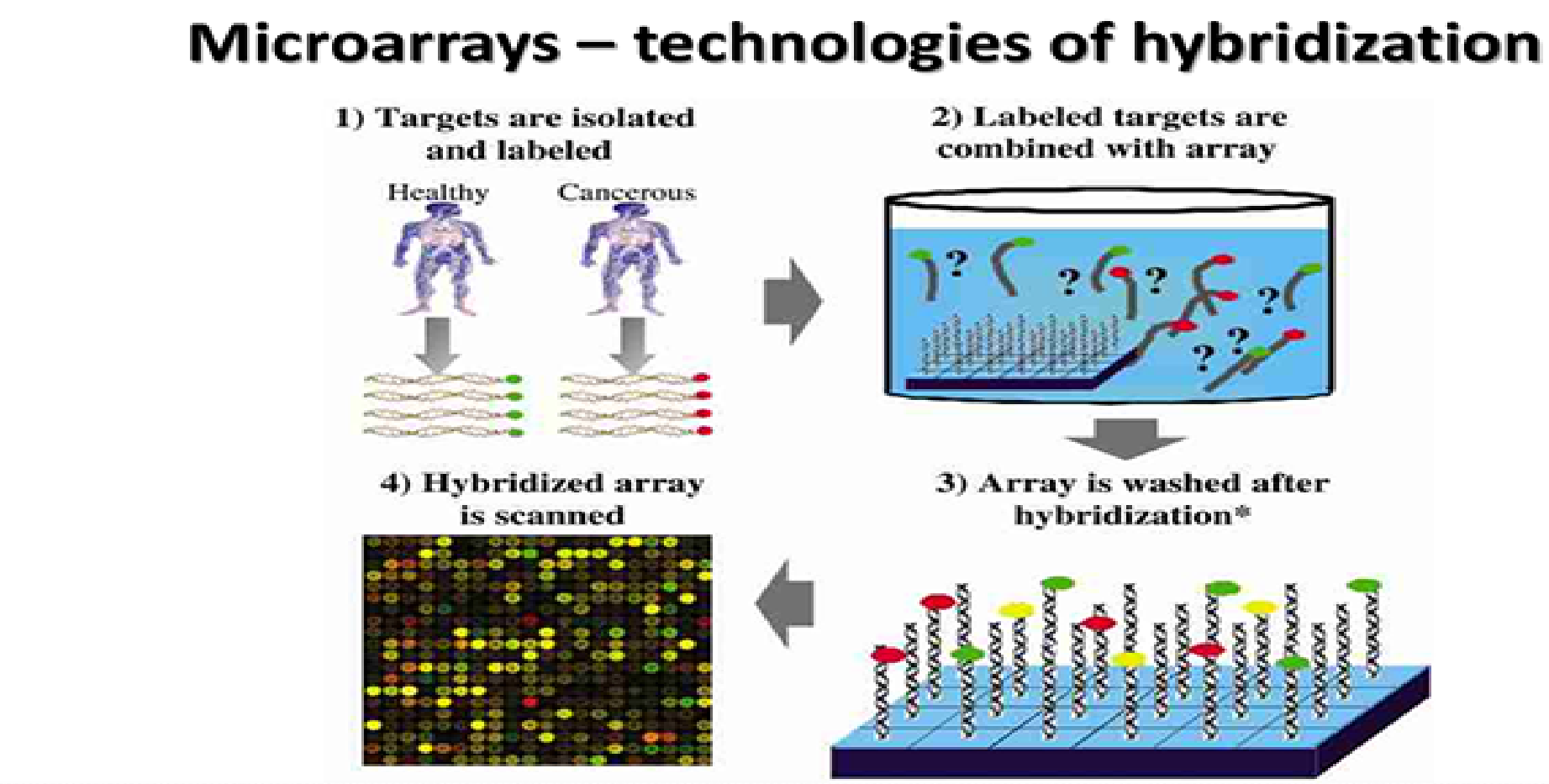
Lithographic masks and de-protection through illumination



Digital micromirror device (DMD)



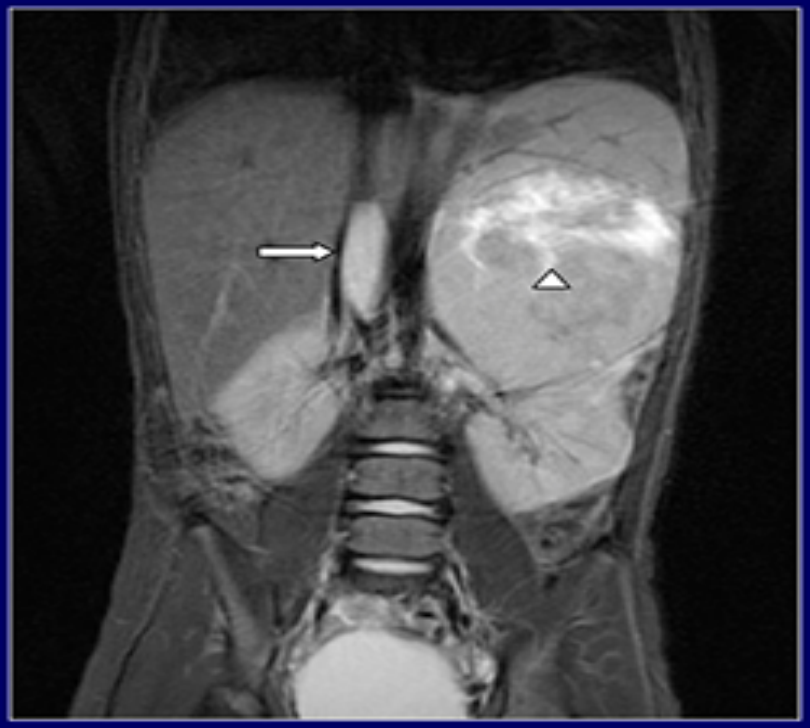
Technologies of hybridization

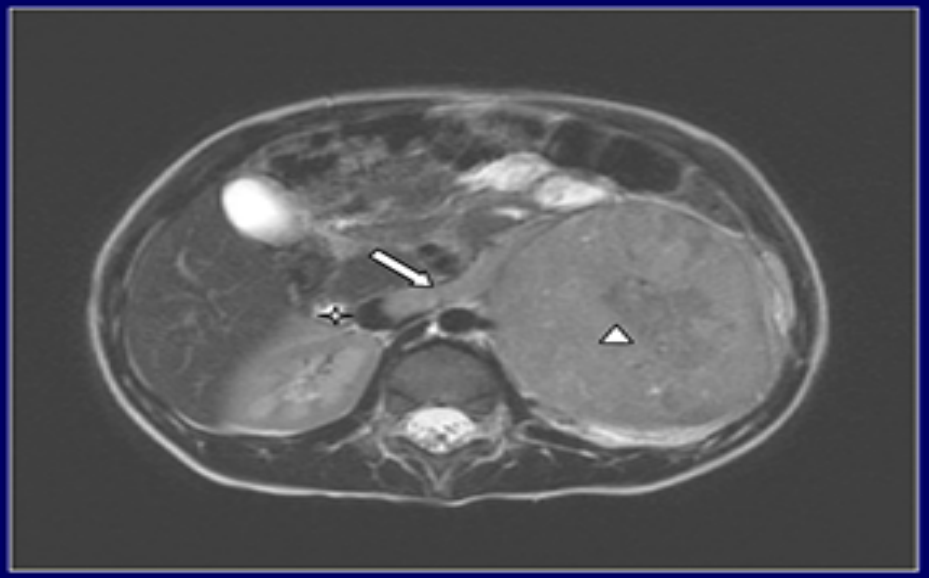


Clinical vignette

Clinical Vignette

MRI:
9 x 8 x 9 cm mass in upper pole left kidney, tumor in Left renal vein and inferior vena cava

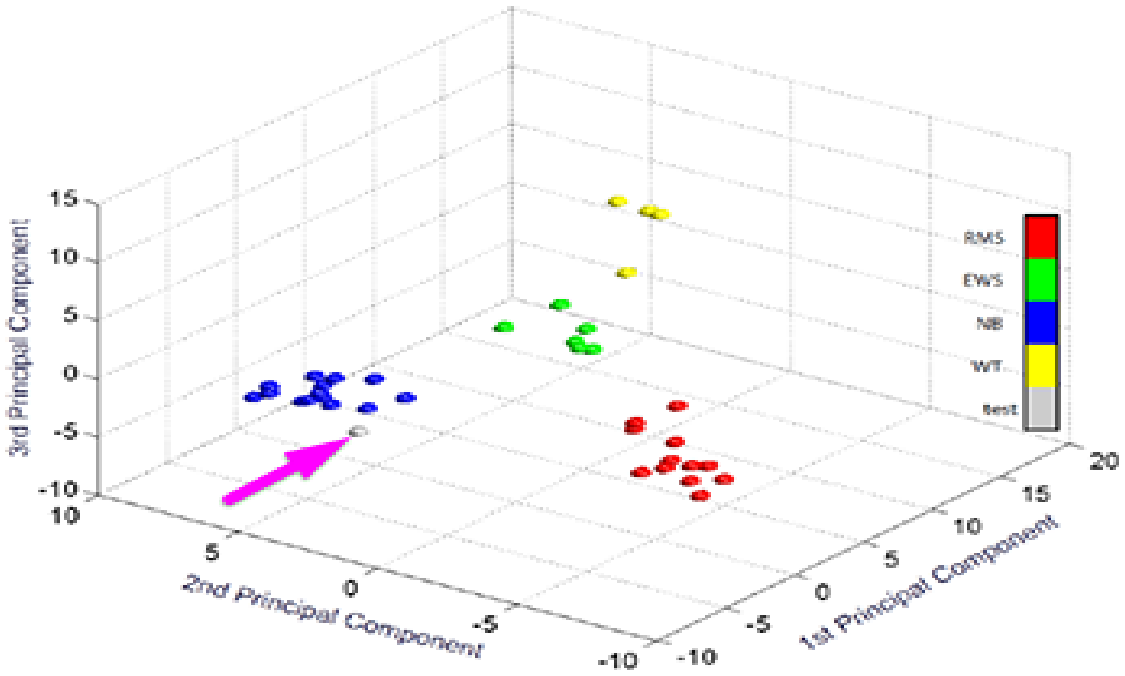




Wilm's tumor?

Cancer diagnosis

Diagnosis of cancers using gene expression profiles derived from DNA microarrays

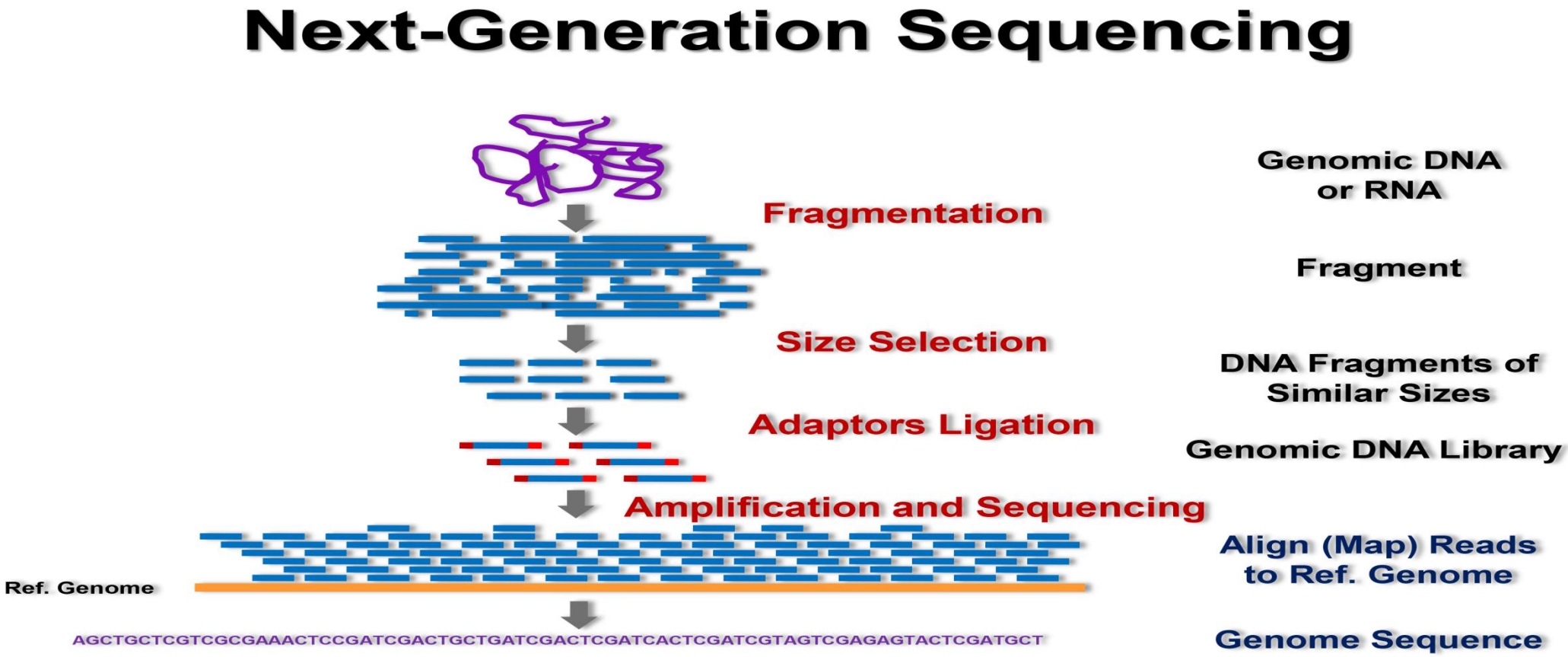


Wilm's tumor

↓

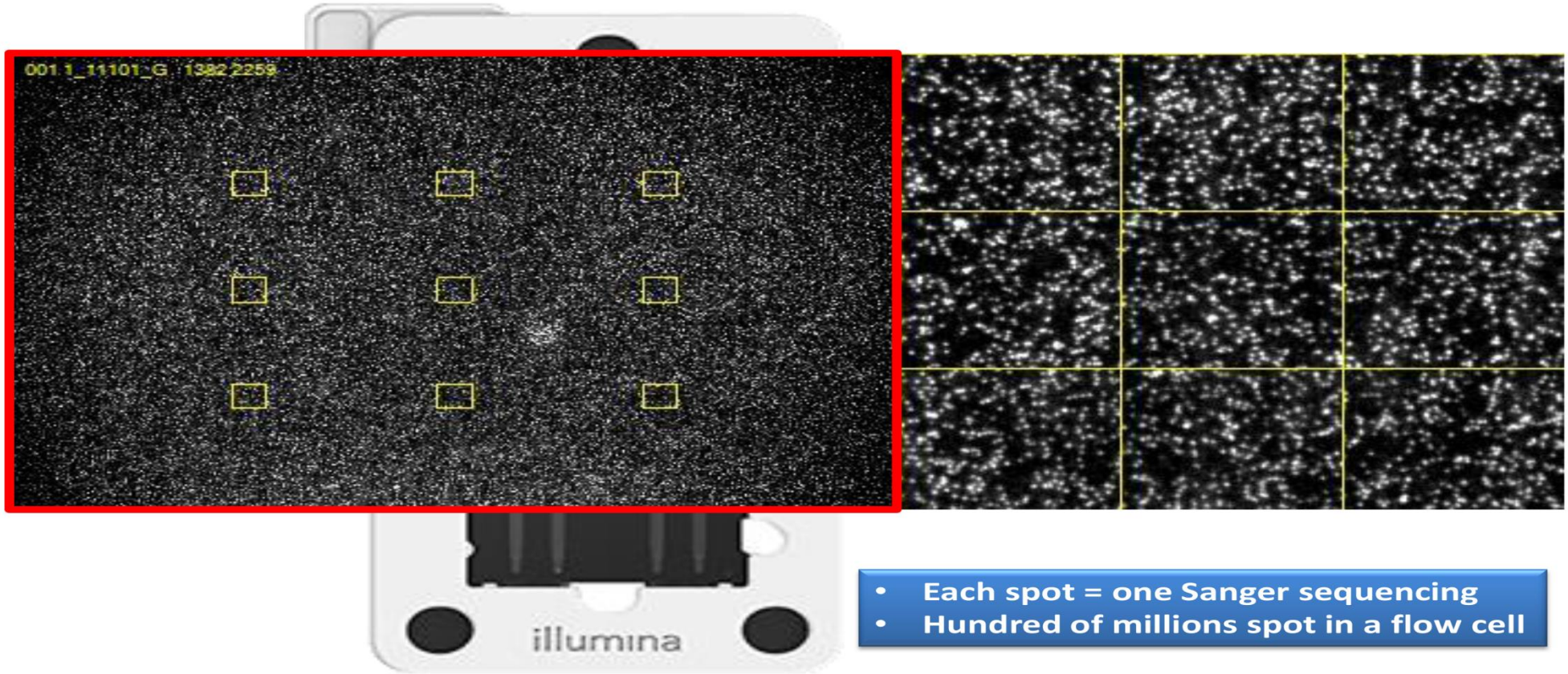
Neuroblastoma

Next-generation sequencing



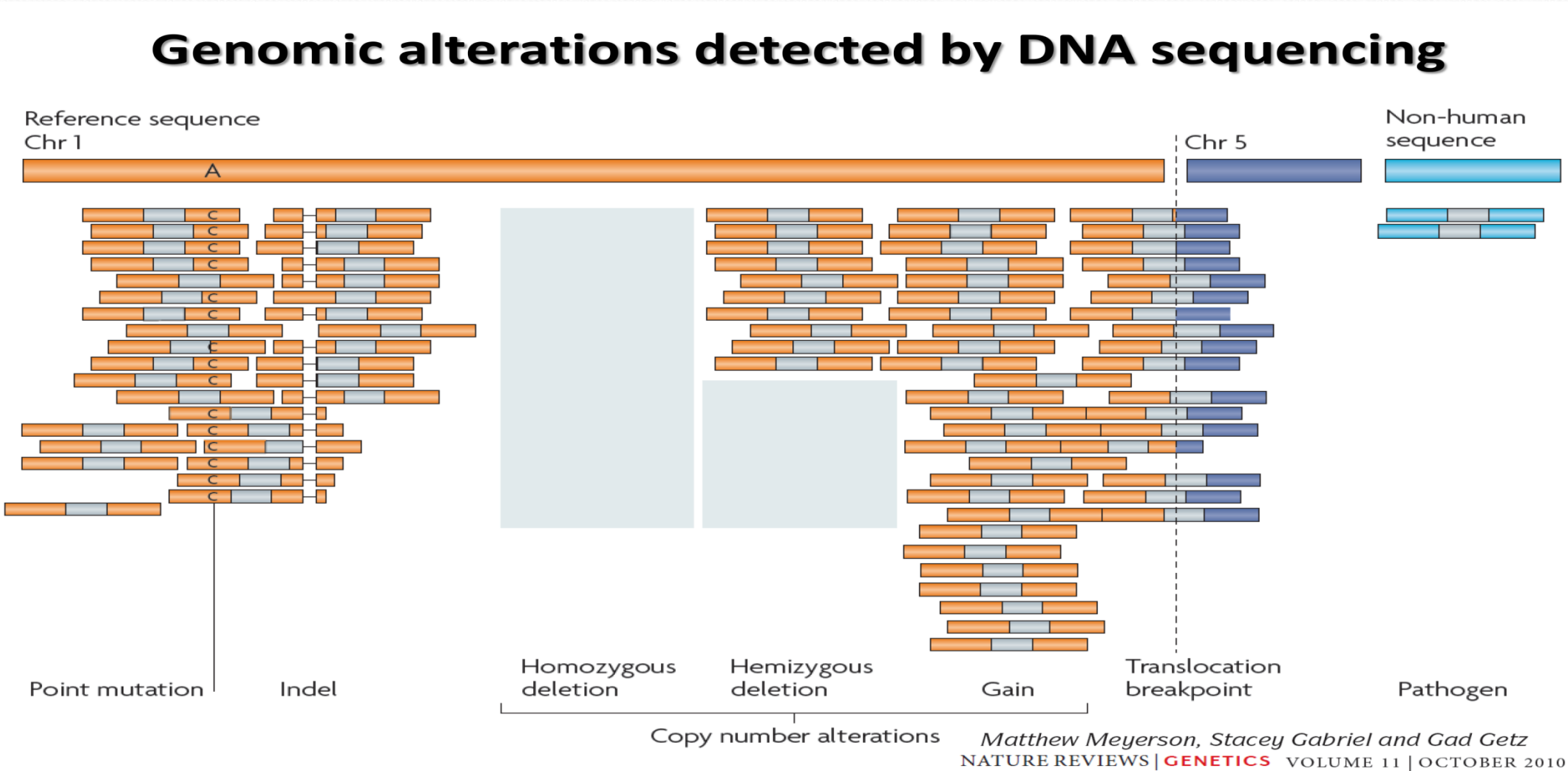
Massively Parallel Sequencing

Massively Parallel Sequencing



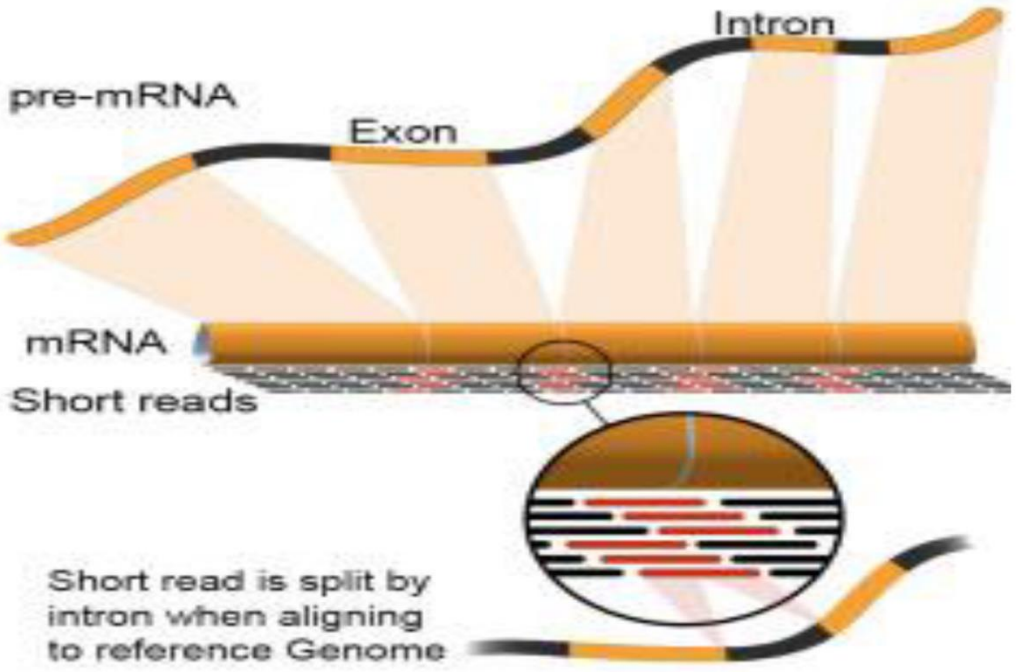
- Each spot = one Sanger sequencing
- Hundred of millions spot in a flow cell

Genomic Alterations



GenomeClinomics for precision medicin

Genomic Alterations Detected by RNA Transcriptome Sequencing



- Digital Gene Expression
- Expressed Mutations
- Alternative Splicing Events
- Expressed Fusion Transcripts
- RNA editing
- Novel Transcripts
- Non-coding RNAs

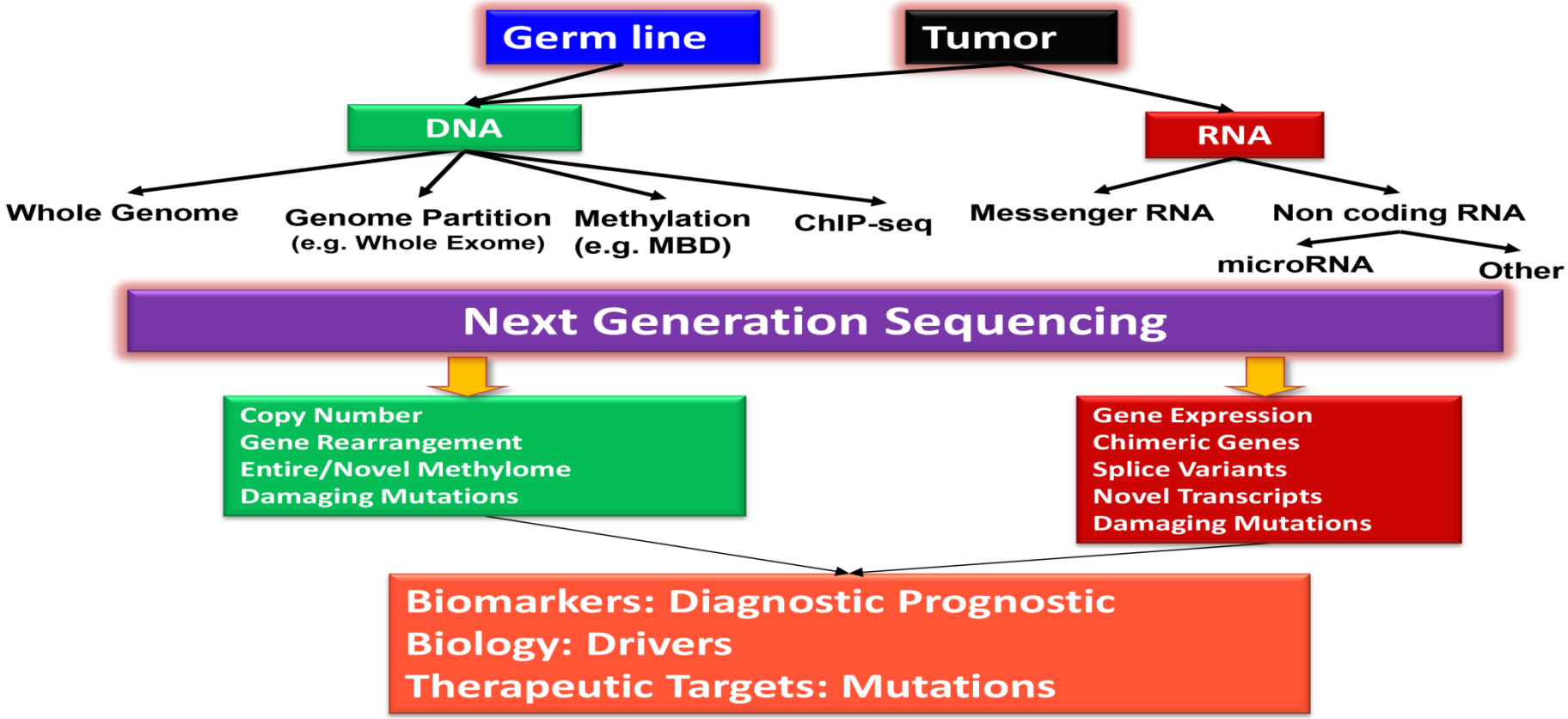
Properties

Properties of the next-generation sequencing technologies

- No need to prepare clones for DNA fragments
- No need of prior knowledge for probe design
- Able to detect balanced genome structure changes
- Parallel sequencing at basepair resolution– massive-throughput (up to 100s Gb/run)
- Cheaper (per nucleotide) and faster per genome

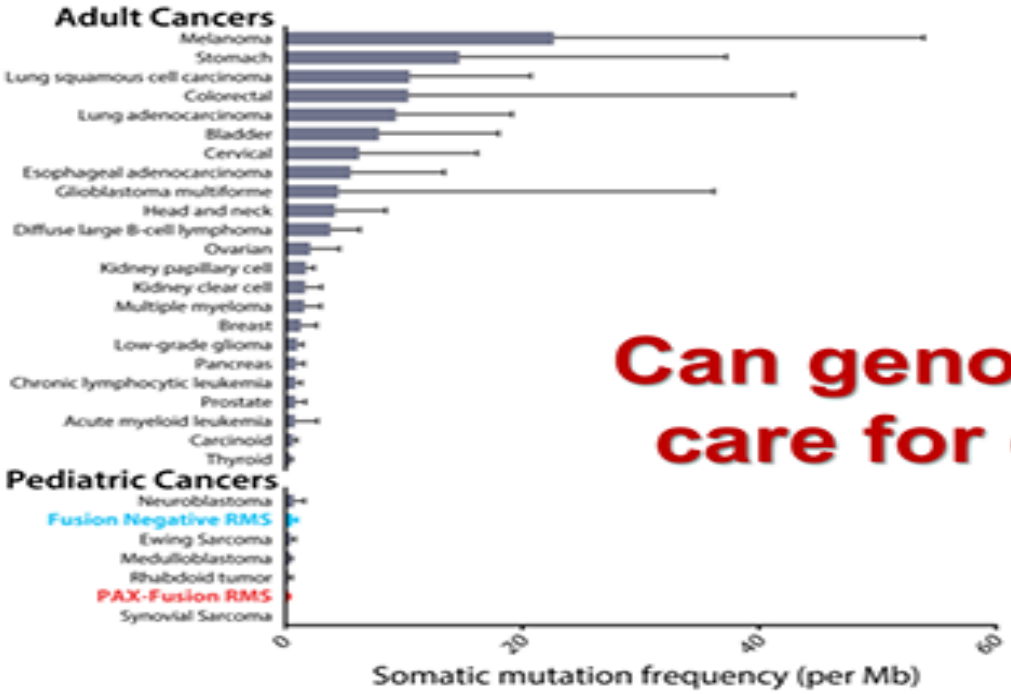
Cancer Genomes

Next Generation Sequencing Allows for Comprehensive Analysis of Cancer Genomes on the Same Platform



Pediatric cancer mutations

Pediatric Cancers Have A Low Number of Somatic and Actionable Mutations At Initial Diagnosis



Can genomics help clinical care for cancer patients?

2014, *Cancer Discovery*, Shern, J. F. *et al.*, Comprehensive Genomic Analysis of Rhabdomyosarcoma 2013, *Nature*, Lawrence, M. S. *et al.*, Mutational heterogeneity in cancer

ClinOmics Program

ClinOmics Program – Multidimensional Integrated Clinical Omics Platform for all patients at CCR

Personalized Medicine and Imaging

Clinical Cancer Research

MultiDimensional ClinOmics for Precision Therapy of Children and Adolescent Young Adults with Relapsed and Refractory Cancer: A Report from the Center for Cancer Research

Wendy Chang[1,2,3], Andrew S. Brohl[1,4], Rajesh Patidar[1], Sivasish Sindiri[1], Jack F. Shern[1,2], Jun S. Wei[1], Young K. Song[1], Marielle E. Yohe[1,2], Berkley Gryder[1], Shile Zhang[1], Kathleen A. Calzone[5], Nityashree Shivaprasad[1], Xinyu Wen[1], Thomas C. Badgett[1,6], Markku Miettinen[7], Kip R. Hartman[8,9], James C. League-Pascual[2,8], Toby N. Trahair[10], Brigitte C. Widemann[2], Melinda S. Merchant[2], Rosandra N. Kaplan[2], Jimmy C. Lin[1], and Javed Khan[1]

Clin Cancer Res. 2016 Aug 1;22(15):3810-20

Study design

Study Design

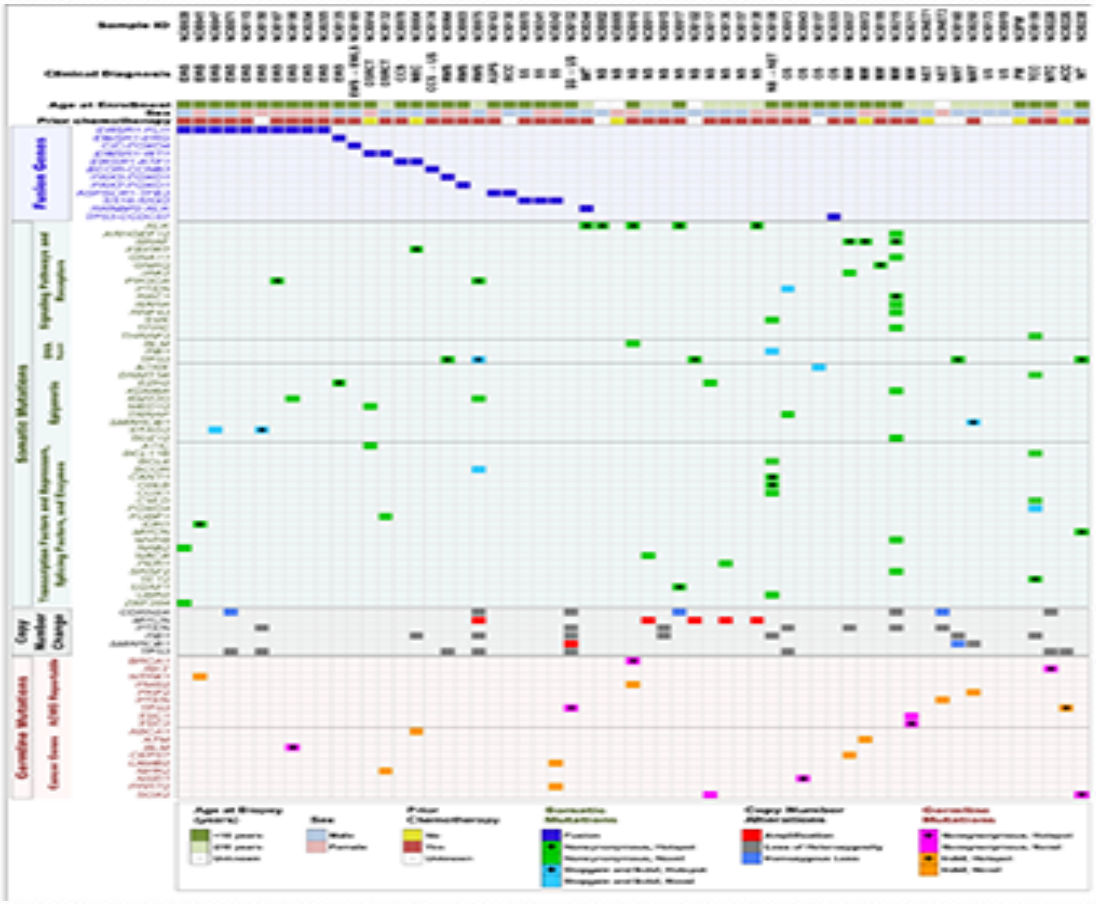
- Pilot study to determine the utility and feasibility of performing comprehensive genomic analyses to identify clinically actionable mutations in pediatric and young adult patients with metastatic, refractory or relapsed solid tumors
- 59 patients enrolled to Omics protocol (10-C-0086) at the Pediatric Oncology Branch, Center for Cancer Research (CCR), NCI (2010-2014)
- Age 7 months-25 years
- 20 diagnostic categories (non-CNS, solid tumors)
- Comprehensive multi-omics exome germline & tumor, RNAseq tumor & Illumina Omni SNP arrays of tumor

Definitions: Actionable

Definitions: Actionable

- **Actionable germline mutation:** loss of function mutation or known hotspot activating mutation of a cancer consensus gene or pathogenic or likely pathogenic mutation of an American College of Medical Genetics (ACMG) Gene
- **Actionable somatic mutation:** genomic alterations that changes the patient's diagnosis, or may be targeted with FDA approved drugs or in the context of existing clinical trials according to the NCI-adult MATCH-Criteria

Integrated landscape



Multi-Omics Integrated Landscape

RNAseq
Diagnostic, Driver, Actionable

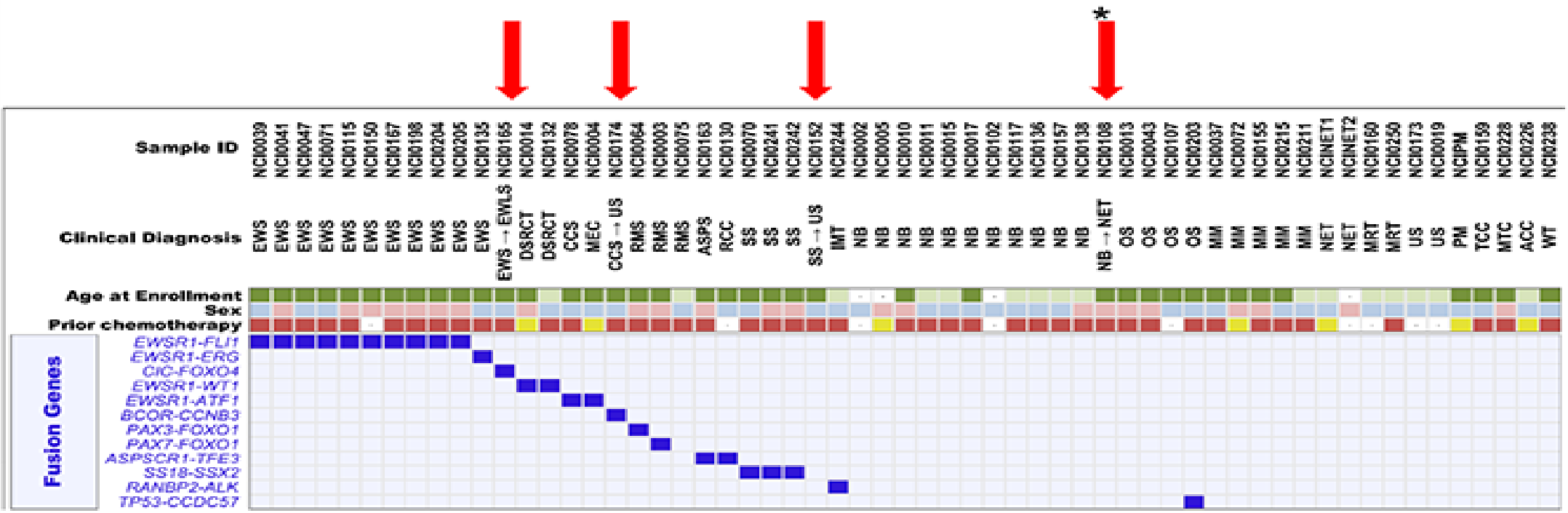
DNAseq and RNAseq
Somatic: Driver, Actionable

DNA copy number & RNAseq
Somatic: Driver, Actionable

DNAseq
Germ line: Disease causing, Actionable

Fusion genes

Presence or absence of fusion genes and/or expression profiles confirms diagnosis or leads to revision of diagnosis



Pediatric germline mutations

~10% of Pediatric and Adolescent Young Adults with Cancers have Actionable Germline Mutations

Table 1. Germline mutations in American College of Medical Genetics (ACMG) reportable genes and tumor suppressor genes identified in 7 patients

Sample	Diagnosis	Gene	Mutation	Disease	Hotspot	Notes	Reportable by Strict ACMG Criteria
NCI0072	MM	*ATM*	p.Y380fs	Ataxia-Telangiectasia and cancer predisposition syndrome	No	Frameshift insertion of tumor suppressor gene	Yes
NCI0010	NB	*BRCA1*	Q1313X	Hereditary breast and ovarian cancer syndrome	Yes	Pathogenic, reportable	Yes
NCI0010	NB	*PMS2*	p.K356fs	Lynch syndrome and mismatch repair cancer syndrome	No	Frameshift deletion of tumor suppressor gene	Yes
NCINET2	NET	*PTEN*	p.R14fs	PTEN Hamartoma tumor syndrome	No	Frameshift deletion of tumor suppressor gene	Yes
NCI0228	MTC	*RET*	M918T	Multiple endocrine neoplasia 2B	Yes	Pathogenic, reportable	Yes
NCI0152	SS → US	*TP53*	R175H	Li-Fraumeni syndrome	Yes	Patient tumor has LOH of wild-type tp53 on other allele	No
NCI0226	ACC	*TP53*	A159K	Li-Fraumeni syndrome	Yes	Tumor has LOH of wild-type tp53 on other allele, novel 2 base non-frameshift substitution, c.358_359delGCinsTT	No
NCI0211	MM	*TSC1*	p.S828R	Tuberous sclerosis type 1, lymphangioleiomyomatosis, focal cortical dysplasia, and everolimus sensitivity	No	Nonsynonymous SNV, autosomal dominant, patient also has a germline TSC2 mutation	No
NCI0211	MM	*TSC2*	p.T246A	Tuberous sclerosis type 2, and lymphangioleiomyomatosis	Yes	Nonsynonymous SNV, autosomal dominant, patient also has a germline TSC1 mutation	No

NOTE: Mutations were confirmed by direct visualization on an IGV viewer, and by Sanger sequencing.
Abbreviations: ACC, adrenocortical carcinoma; MM, malignant melanoma; MTC, medullary thyroid carcinoma; NET, neuroendocrine tumor; RMS, rhabdomyosarcoma; SS, synovial sarcoma; US, undifferentiated sarcoma; horizontal arrow indicates change in diagnosis.

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Actionable somatic mutations

Approximately 50% (30/59) of Pediatric and Adolescent Young Adults with Cancers Have Actionable Somatic Mutations

Table 2. Summary of actionable mutations in relapsed and refractory pediatric solid tumors

Sample	Diagnosis	Gene	Stage	Modality	Mutation	AA Change	Level	Drug	Clinical trial: Pediatric	FDA-Approval in adults	Exact mutation vs. hotspot	Reference preclinical data for level 3
NCI0037	MM	*BRAF*	Relapsed	WES/WTS	NS SNV	p.V600E	1	Vemurafenib, dabrafenib	Yes	Yes	Exact	—
NCI0072	MM	*BRAF*	Diagnostic	WES/WTS	NS SNV	p.V600E	1	Vemurafenib, dabrafenib	Yes	Yes	Exact	—
NCI0215	MM	*BRAF*	Relapsed	WES/WTS	NS SNV	p.V600E	1	Vemurafenib, dabrafenib	Yes	Yes	Exact	—
NCI0155	MM	*GNAQ*	Relapsed	WES/WTS	NS SNV	p.Q209L	1	Temsirolimus, trametinib, vorinostat	No	Yes	Exact	—
NCI0002	NB	*ALK*	—	WES/WTS	NS SNV	p.R1275Q	2a	Crizotinib	Yes	Yes	Exact	—
NCI0010	NB	*ALK*	Relapsed	WES/WTS	NS SNV	p.F1174V	2a	Crizotinib	Yes	Yes	Exact	—
NCI0017	NB	*ALK*	Relapsed	WES/WTS	NS SNV	p.F1174L	2a	Crizotinib	Yes	Yes	Exact	—
NCI0138	NB	*ALK*	Relapsed	WES/WTS	NS SNV	p.Y1278S	2a	Crizotinib	Yes	Yes	Exact	—
NCI0244	IMT	*ALK*	Relapsed	WTS	*RANBP2-ALK* fusion	—	2a	Crizotinib	No	Yes	Exact	—
NCI0244	IMT	*ALK*	Relapsed	WES/WTS	NS SNV	p.R17T	2a	Ceritinib	No	Yes	Exact	—
NCI0215	MM	*GNA11*	Relapsed	WES/WTS	NS SNV	p.S268F	2a	Trametinib	No	Yes	—	—
NCI0041	EWS	*EGFR*	Relapsed	WES/WTS	NS SNV	p.R82C	2a	EGFR inhibitors	No	No	Exact	—
NCI0075	RMS	*PIK3CA*	Relapsed	WES/WTS	NS SNV	p.P104Q	2a	PI3K/AKT/mTOR inhibitors	Yes	Yes	Exact	—
NCI0167	EWS	*PIK3CA*	Refractory	WES/WTS	NS SNV	p.D930G	2a	PI3K/AKT/mTOR inhibitors	Yes	Yes	Exact	—
NCI0013	OS	*PTEN*	Relapsed	WES/WTS	Frameshift deletion	p.K80fs	2a	PI3K/AKT/mTOR inhibitors	Yes	No	—	—
NCINET2	NET	*PTEN*	—	WES/WTS	Germline frameshift deletion/somatic LOH	p.R54fs	2a	PI3K/AKT/mTOR inhibitors	Yes	No	—	—
NCI0228	MTC	*RET*	Relapsed	WES/WTS	Germline SNV	p.M918T	2a	Vandetanib	Yes	Yes	Exact	—
NCI0017	NB	*CDKN2A*	Relapsed	SNP Array/WTS	Homozygous loss	—	3	CDK4/6 inhibitor	No	No	—	56
NCI0071	EWS	*CDKN2A*	Relapsed	SNP Array/WTS	Homozygous loss	—	3	CDK4/6 inhibitor	No	No	—	56
NCINET2	NET	*CDKN2A*	—	SNP Array/WTS	Homozygous loss	—	3	CDK4/6 inhibitor	No	No	—	56
NCI0011	NB	*MYCN*	Relapsed	SNP Array/WTS	Amplification	—	3	Bromodomain inhibitors	No	No	—	57
NCI0075	RMS	*MYCN*	Relapsed	SNP Array/WTS	Amplification	—	3	Bromodomain inhibitors	No	No	—	57
NCI0102	NB	*MYCN*	—	SNP Array/WTS	Amplification	—	3	Bromodomain inhibitors	No	No	—	57
NCI0136	NB	*MYCN*	Relapsed	SNP Array/WTS	Amplification	—	3	Bromodomain inhibitors	No	No	—	57
NCI0138	NB	*MYCN*	Relapsed	SNP Array/WTS	Amplification	—	3	Bromodomain inhibitors	No	No	—	57
NCI0158	WT	*MYCN*	Relapsed	WES/WTS	NS SNV	p.P44L	3	Bromodomain inhibitors	No	No	—	57, 58
NCI0160	MRT	*SMARCB1*	—	SNP Array/WTS	Homozygous loss	—	3	EZH2 inhibitors	No	No	—	59, 60
NCI0250	MRT	*SMARCB1*	Refractory	WES/WTS	NS SNV	p.R40X	3	EZH2 inhibitors	No	No	—	59, 60
NCI0047	EWS	*STAG2*	Relapsed	WES/WTS	NS SNV	p.E986X	3	PARP inhibitors	Yes	No	—	61
NCI0150	EWS	*STAG2*	—	WES/WTS	NS SNV	p.R29GX	3	PARP inhibitors	Yes	No	Hotspot	61
NCI0211	MM	*TSC1*	Relapsed	WES/WTS	NS SNV	p.S828R	3	Everolimus	No	Yes	—	62
NCI0211	MM	*TSC2*	Relapsed	WES/WTS	NS SNV	p.T246A	3	Everolimus	No	Yes	—	62

NOTE: SNVs were confirmed by direct visualization on an IGV viewer, and validation by Sanger sequencing or confirmation CLIA-certified laboratories.
Abbreviations: EWS, Ewing sarcoma; IMT, epithelioid inflammatory myofibroblastic sarcoma; MM, malignant melanoma; MRT, malignant rhabdoid tumor; MTC, medullary thyroid carcinoma; NB, neuroblastoma; NET, neuroendocrine tumor; OS, osteosarcoma; RMS, rhabdomyosarcoma; WT, Wilms tumor.

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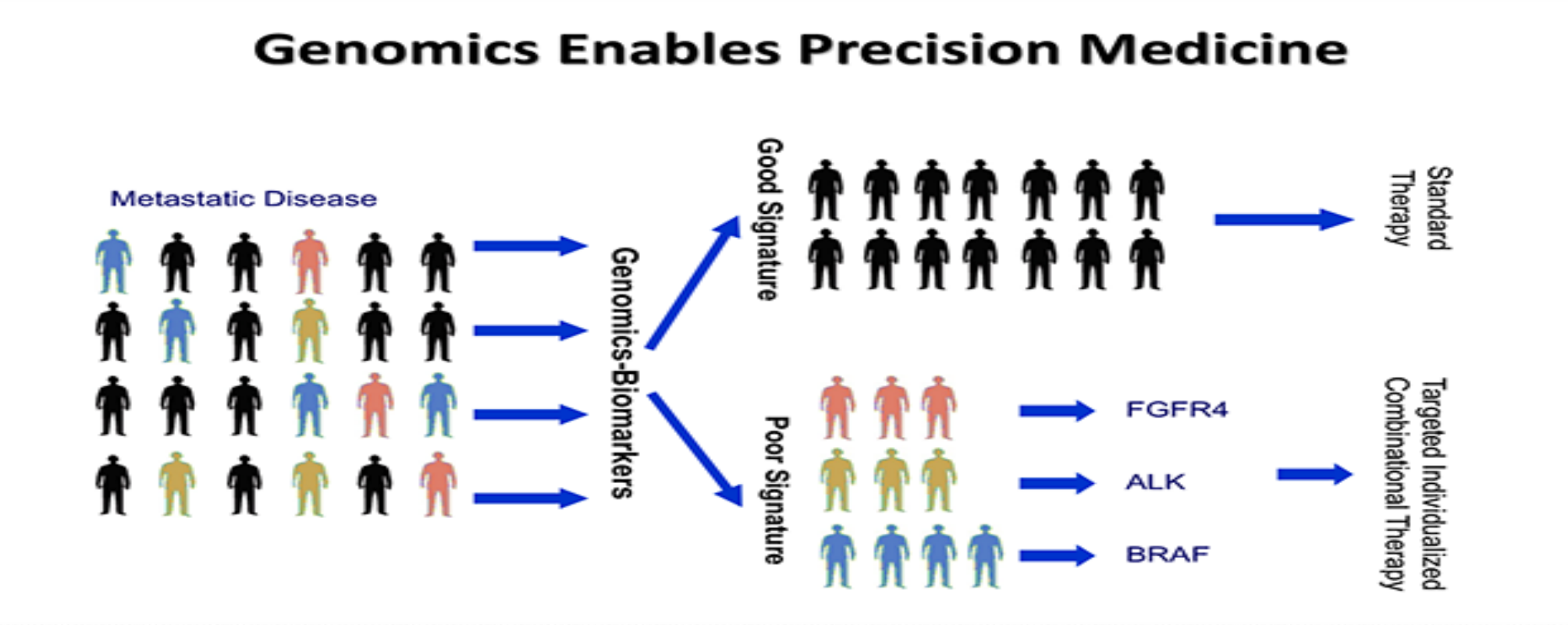
Summary

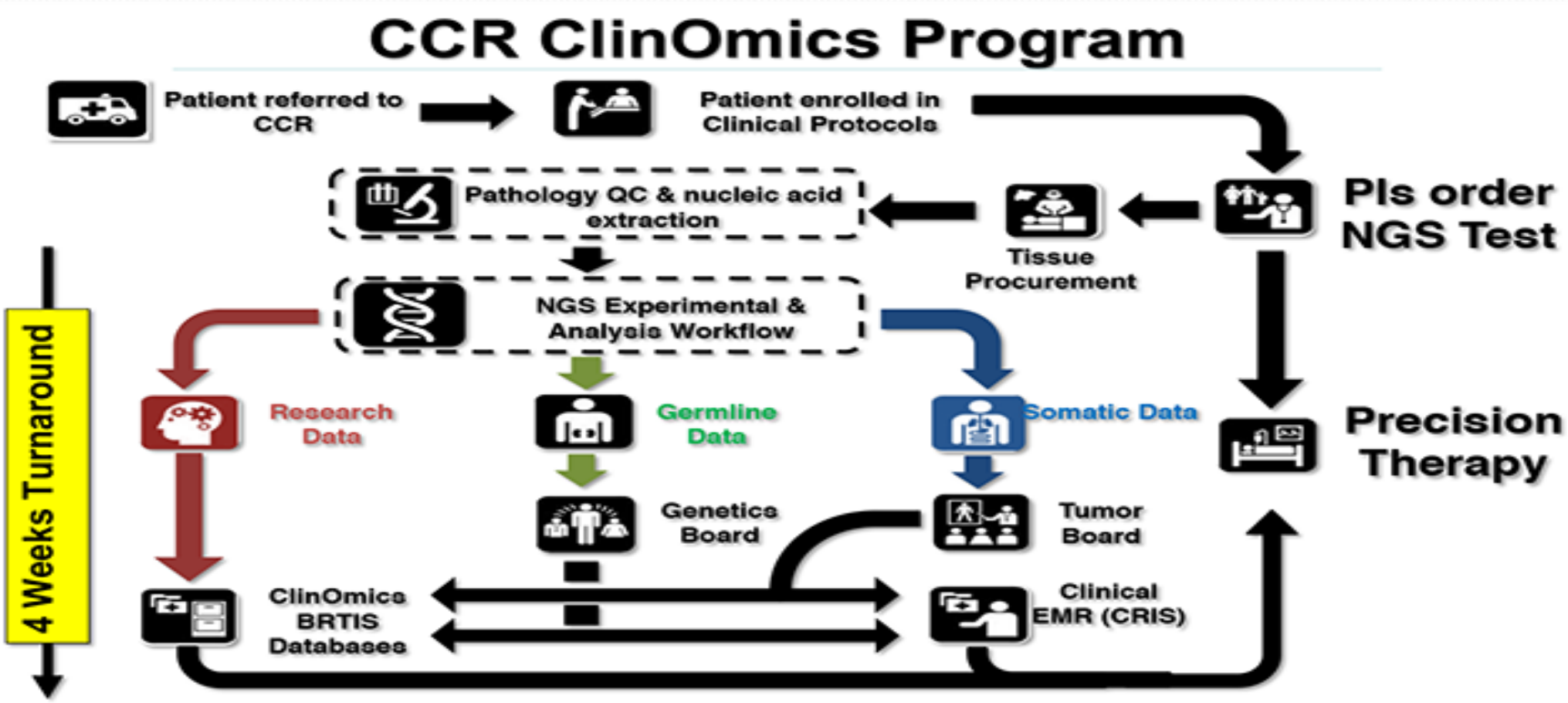
Summary

- Demonstrated the importance and feasibility of performing multi-dimensional ClinOmics in the clinical setting in real time
- ~50% of children with pediatric or AYA patients with relapsed or refractory cancers have actionable somatic mutations
- ~ 10% have actionable germline mutations. Importance of performing parallel germline sequencing; some therapeutically actionable (e.g. DNA repair, PTEN, TSC1, TSC2, HRAS, RET, ALK)

Precision medicine



ClinOmics Program



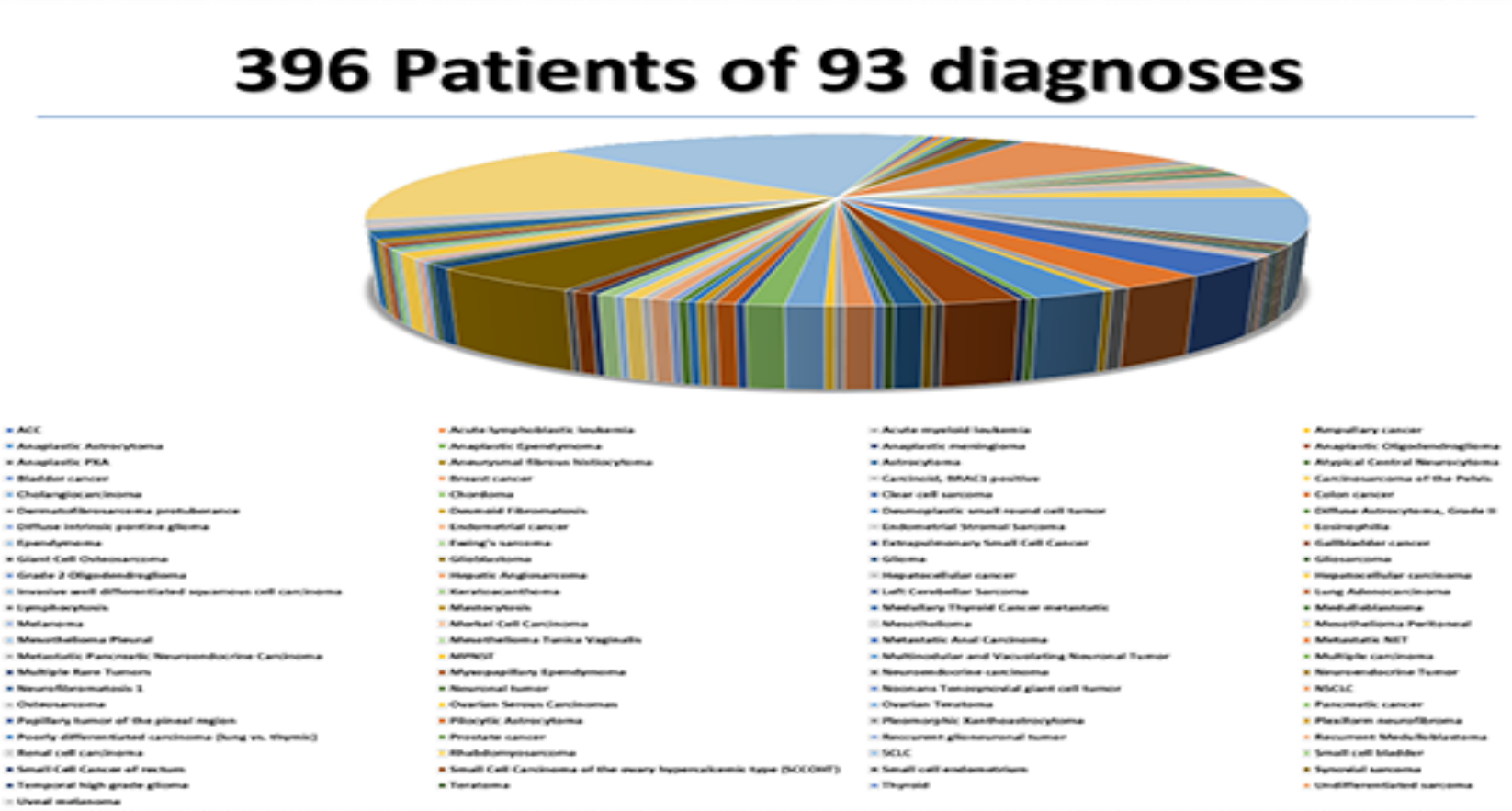
Sequencing equipment

Sequencing Equipment



- Two NextSeq500s for speed and lower throughout
  - 65 Gb/run
  - 14 hours/run
- One HiSeq2500: for high throughput
  - 1000Gb/run
  - 32 exomes or transcriptomes
  - 14 days/run

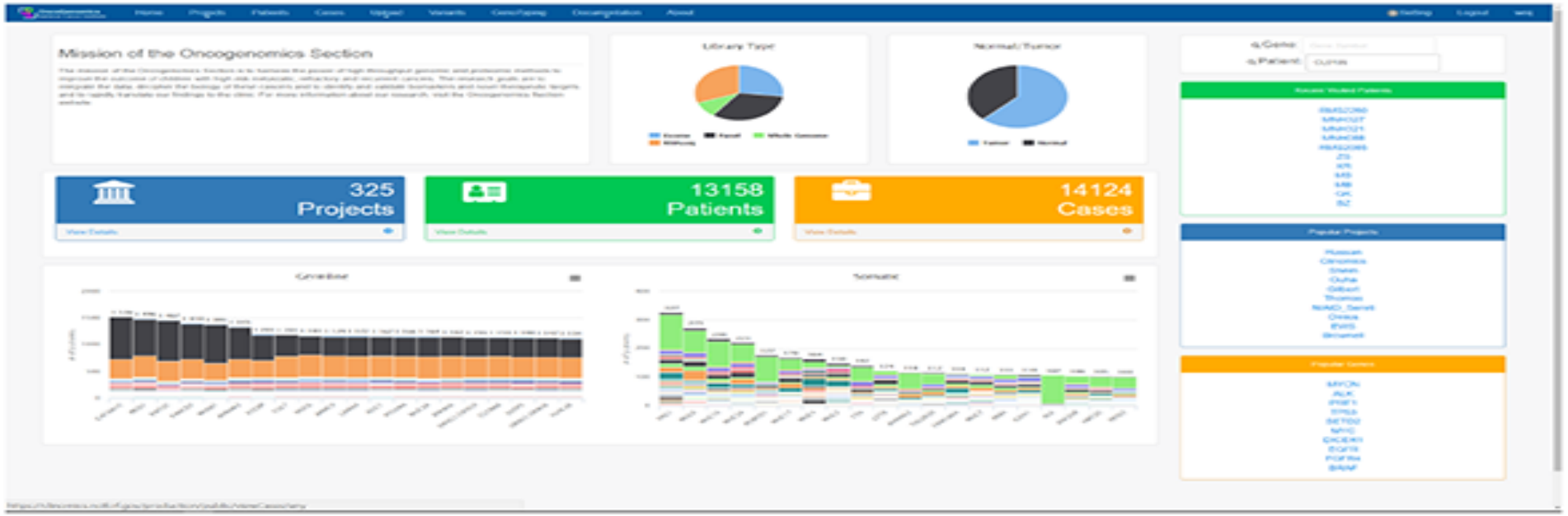
Patient diagnoses



Data portal

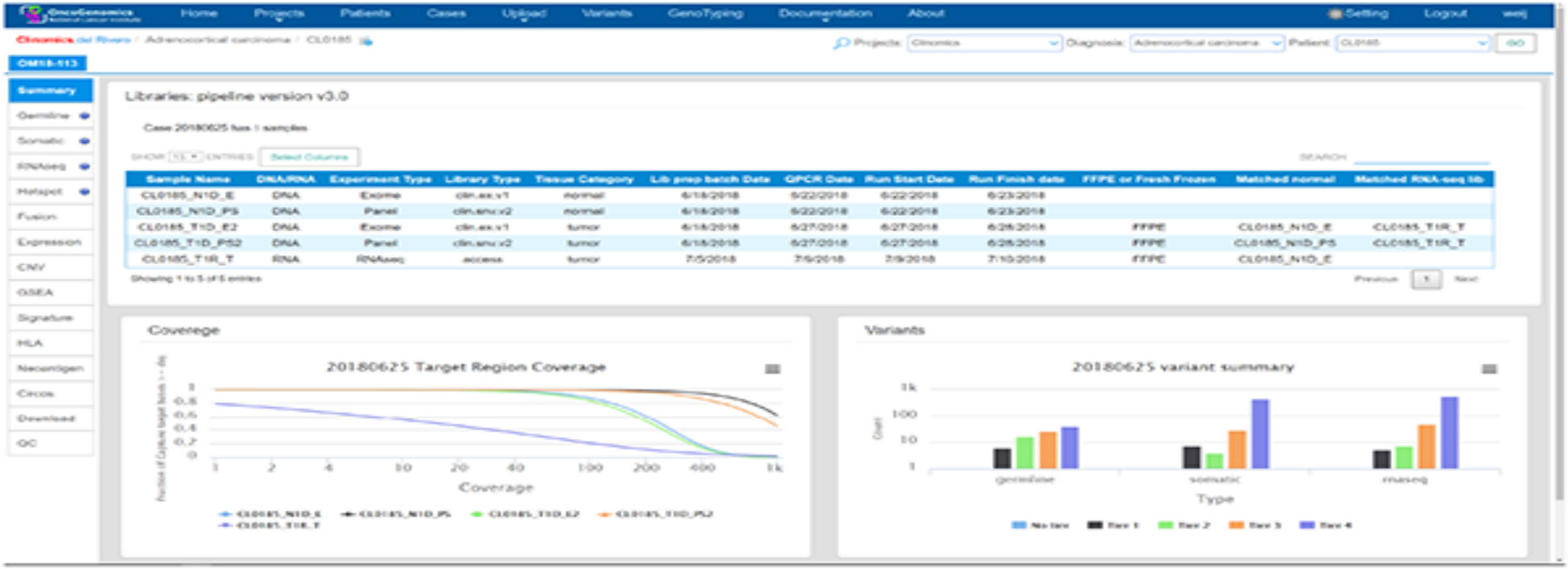
ClinOmics Data Portal

https://clinomics.ncifcrf.gov/production/public/

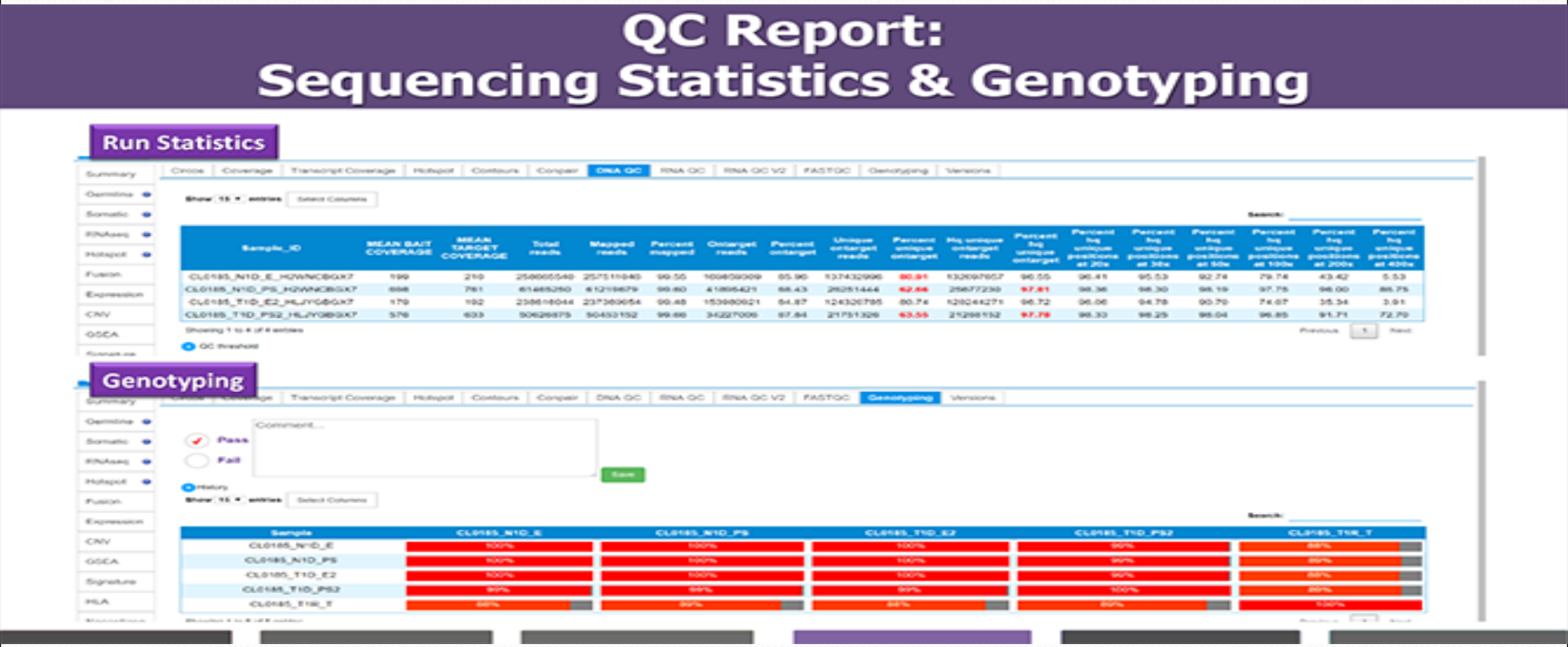


Patient summary

Patient Summary Page



QC report

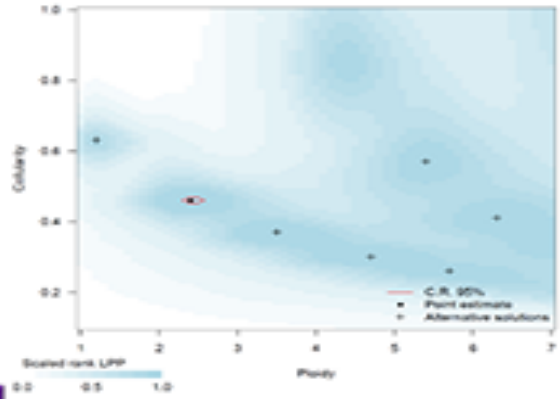

QC Report:
Sequencing Statistics & Genotyping
Run Statistics
Genotyping

Coverage

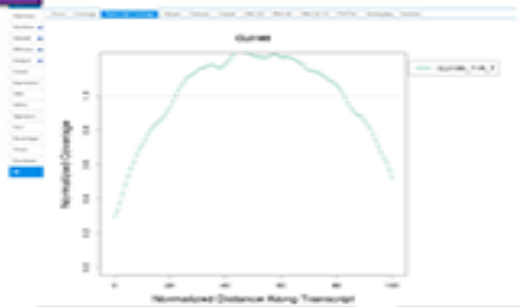
QC Report: Coverage

Circos

Tumor Content



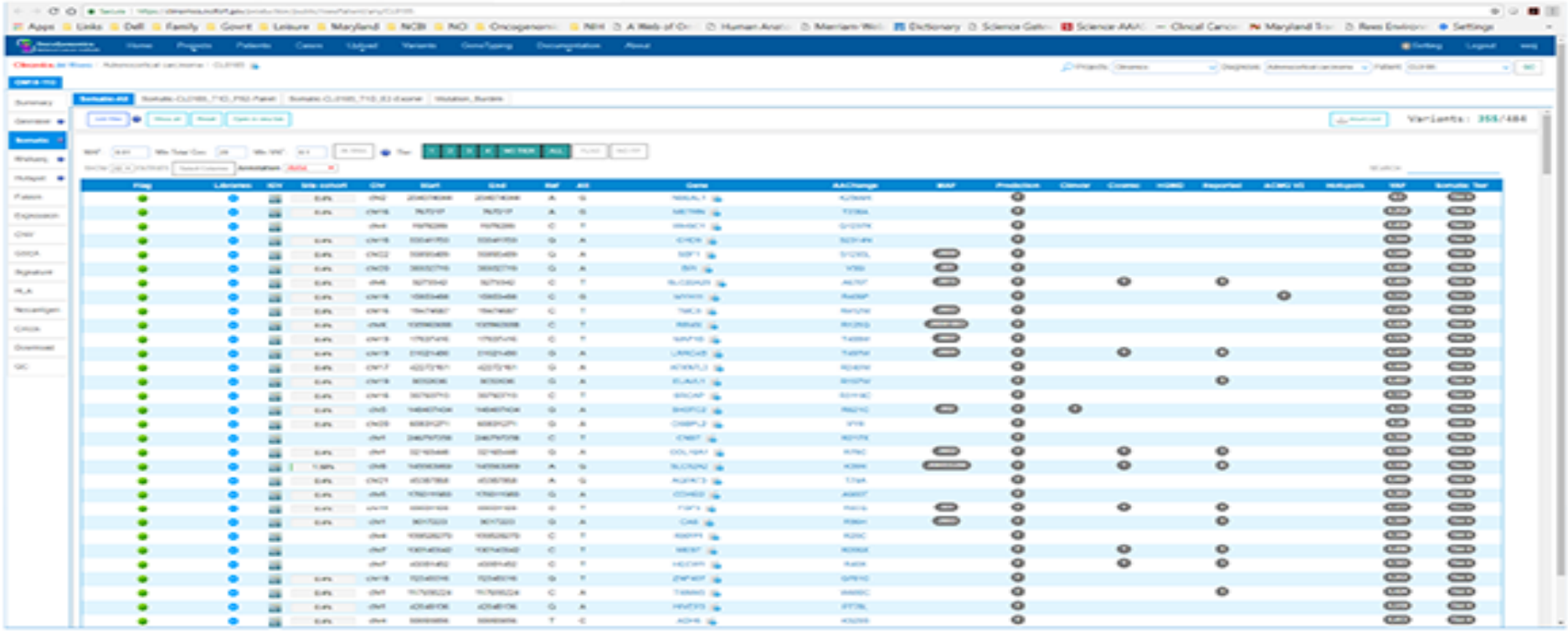
RNA Coverage



Hotspot Coverage

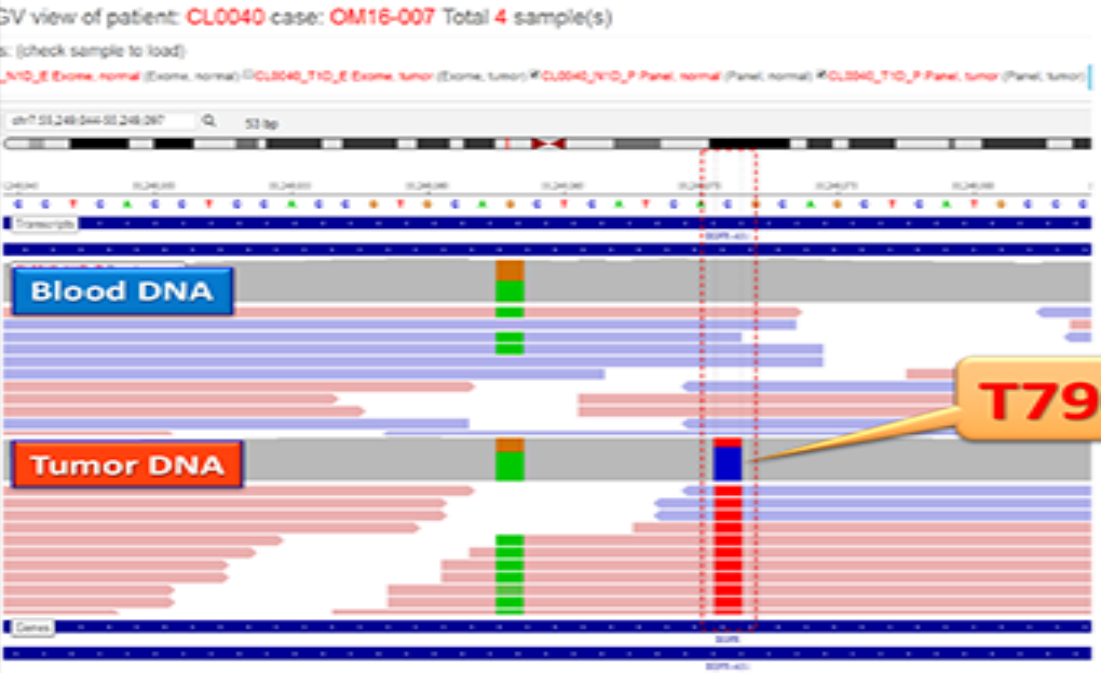
Mutations

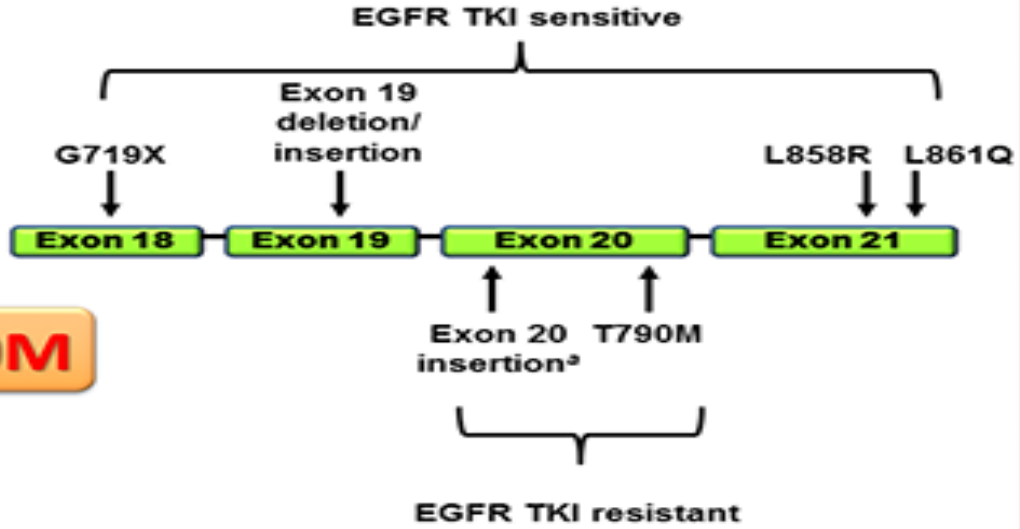
Germline and Somatic Mutations



EGFR mutations

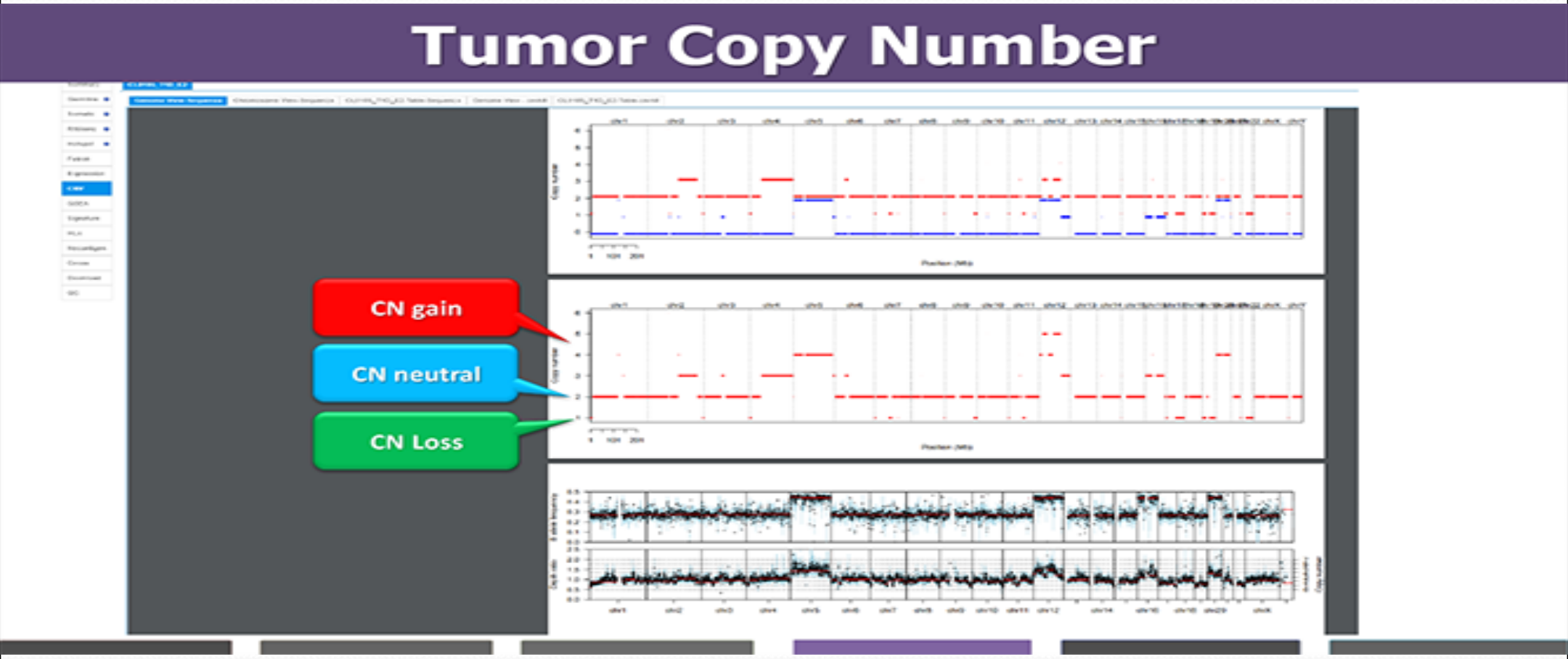
EGFR mutations in NSCLC





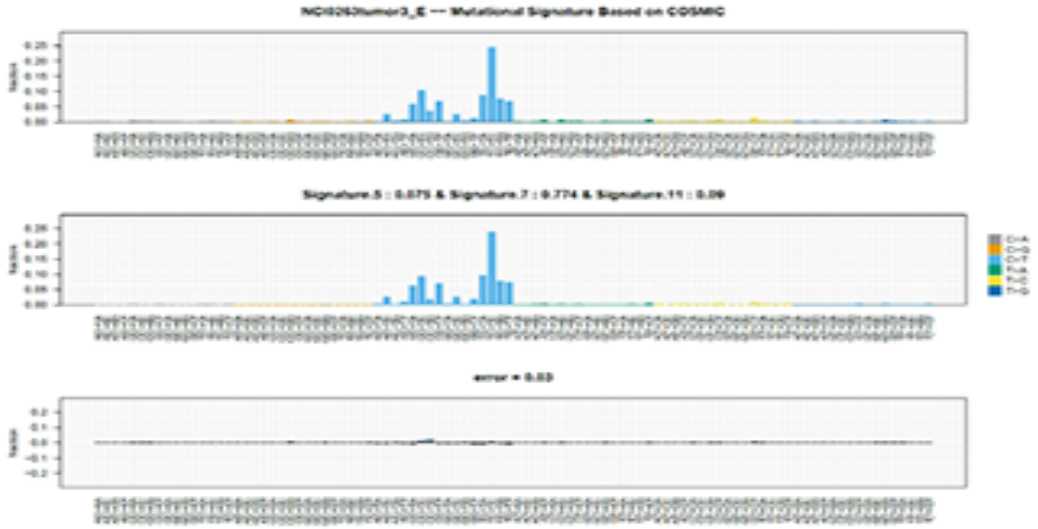
https://www.mycancergenome.org/content/disease/lung-cancer/egfr/

Tumor copy number

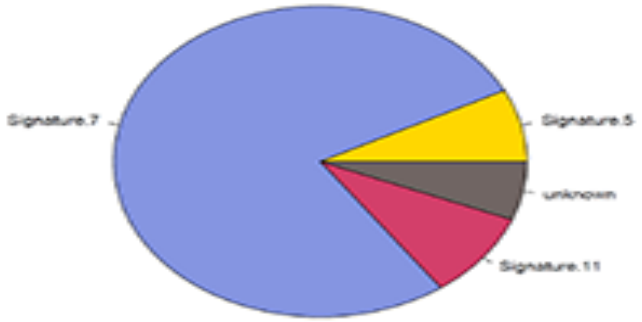


Mutation signatures

Mutation Signatures for Tumor



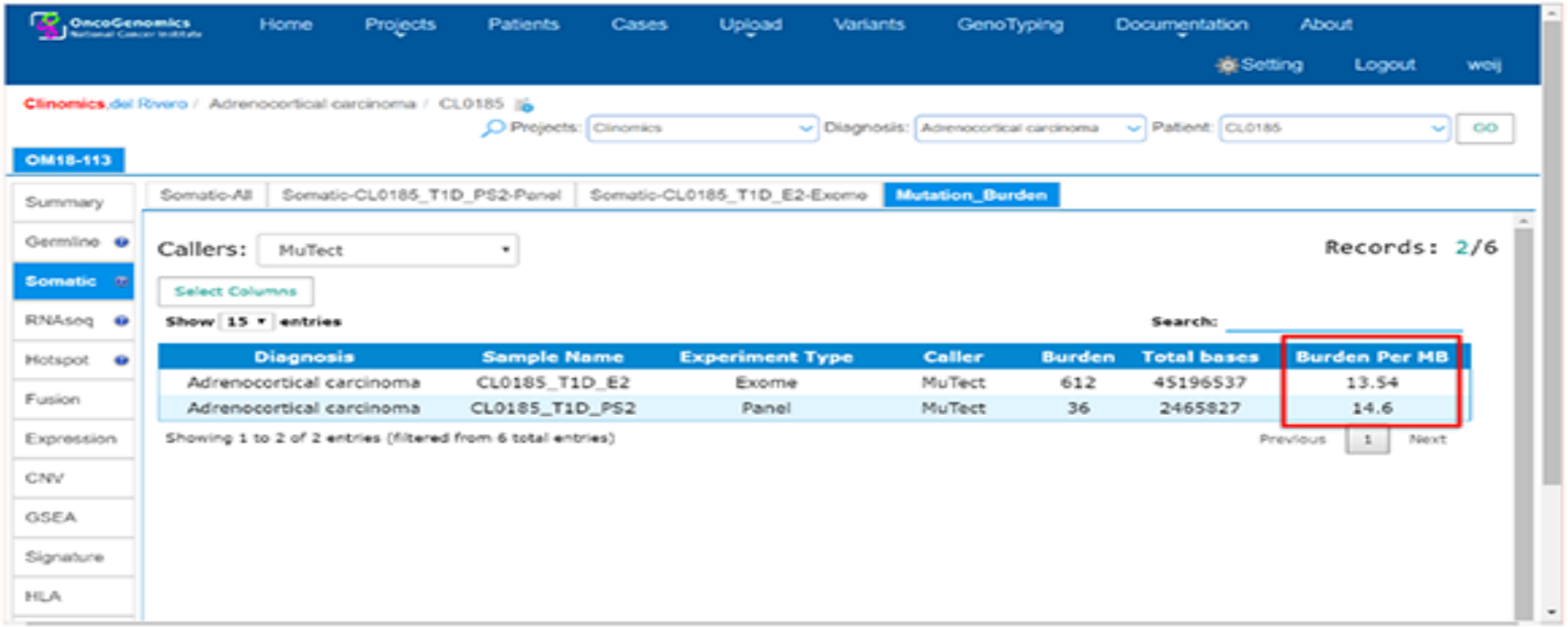
NCI0263: Melanoma



COSMIC (https://cancer.sanger.ac.uk/cosmic/signatures)

Signature 7: UV signature

Mutation burden



Fusion gene detection

Fusion Gene Detection from RNA-seq experiments

Genomic information

Other Useful Genomic Information

- HLA typing (Tissue typing)
- Neoantigen prediction
- Gene expression
- Gene Set Enrichment Analysis (GSEA)
- Survival analysis if outcome data is available

Conclusions

Conclusions

- Next generation sequencing (including whole genome, exome, and transcriptome) determines the complete genomic and epigenetic portrait of cancers at the base pair level

- Integrated analyses of the cancer can identify biologically relevant diagnostic, prognostic biomarkers and novel targets for precision medicine

Acknowledgements